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STAPHYLOCOCCAL INFECTIONS

BY

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CONTENTS

*This series of papers is a result of a conference on *Staphylococcal Infections* held by the Section of Biology of The New York Academy of Sciences, February 16 and 17, 1956.

Part IV. Clinical Staphylococcal Infections

INTRODUCTORY REMARKS

By Hilary Koprowski
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In a brilliant essay published recently, Rene J. Dubos commented on the persisting importance of bacterial infections as problems in medicine and public health, even though supposedly effective control measures are the hallmarks of the present era. That this is so is attested by the fact that bacterial infections comprise the subject of monographs based on conferences held twice within a span of 2 weeks at the Academy. This publication deals with *Staphylococcus*, while that shortly to follow it will deal with natural resistance to infections, and will discuss many of the enigmas of nonsusceptibility and susceptibility to bacterial infections. The swing toward devoting more attention to these aspects of microbiology reminds one of the statement of Schopenhauer that "Opinion is like a pendulum and obeys the same law. If it goes past the center of gravity on one side, it must go a like distance on the other; it is only after a certain time that it finds the true point at which it can remain at rest."

The first paper of this monograph deals with "The Problem of Staphylococcal Infection," and the final paper with "The Unknowns of Staphylococcal Infection." The finale, like so many other endings, is but a prelude and stimulus for future investigations. These studies are, slightly paraphrasing A. P. Herbert, "in main, faithful mirrors of our times; and if the reflections displease us . . . no man of sense will throw stones at his looking-glass; he will take steps to improve his reflections."

Part I. Host Factors in Experimental Staphylococcal Infections

THE PROBLEM OF STAPHYLOCOCCAL INFECTIONS

By Walsh McDermott

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The control of the great plagues and certain of the acute bacterial infections have not delivered mankind from infectious disease, but they have very decidedly changed the *nature* of that disease in certain countries of the world including our own.

The principal change in these countries has been the throwing into prominence of those bacterial diseases that characteristically require a relatively greater reduction of bodily defense before infection can become disease. To be sure, the initiation of any disease involving a particular microbe presumably requires some breakdown, however temporary, of the defense mechanisms of the host. Nevertheless, certain microbes seem to require more of such help than others.

As we all know, the 3 principal diseases involving bacteria that are most prominent in the United States are those diseases produced by the bowel flora, by tubercle bacilli, and by staphylococci. These 3 groups of diseases have a number of features in common. Above all, they share in the fact that harboring of the parasite is commonplace but disease is rare. This means that the spread of these diseases among a group of persons is not to be regarded as a visitation from without but rather as an indication that something is going wrong with the crude equilibrium that had previously existed in that community.

If this be so, it follows that we should not be exclusively preoccupied with attempts to drive invaders out, for we are not really dealing with invaders but with original settlers. If they were not actually here first, they were certainly a close second. Indeed, in the specific case of staphylococci, most babies acquire them during the first week of life. Our preoccupation should be with attempts to discover what it is that goes wrong and that permits these "hangers-on" to gain control. Or, more specifically, we should be concerned with a study of *what there is* to *go* wrong. The fact that this is indeed a subject of widespread experimental preoccupation is emphasized from the titles listed in this monograph.

Before concentrating exclusively on staphylococci, however, it might be helpful to recall certain of the other properties held in common by staphylococcal disease and its 2 sister diseases, those caused by tubercle bacilli and those caused by individual members of the bowel flora. First, with all 3 groups of diseases, there is the fact mentioned previously that the presence of the microbe is commonplace, while disease and illness are rare. This leads to the obvious inference that either the microbe or the host must undergo some significant alteration before infection can become disease. In addition, and with certain individual exceptions, there run through the microbes of all 3

groups of disease the common tendencies (1) to produce localized lesions with destruction of tissue, (2) to produce endotoxins or tuberculin, (3) to survive intracellularly (only in the case of tubercle bacilli and staphylococci), (4) to produce illness that may range from the exceedingly acute all the way to the barely perceptible chronic forms, and (5) to exist or to emerge in a form resistant to one or more antimicrobial drugs.

For these reasons, and even without respect to the matter of drug resistance, the antimicrobial therapy of these forms of localized bacterial diseases has always represented a less important portion of the total therapy than it has with certain of the acute, relatively nondestructive microbial diseases.

Generalizations such as these are quite helpful when we are trying to outline and define a problem. We all know, however, that these generalizations soon reach their limits and, if there is one thing characteristic of staphylococci, it is the complete *lack* of generality that they display with respect to many of the attributes related to the disease production in which we are most interested.

Although these papers discuss something called staphylococcal infection, we all know that this is not always quite the same thing as staphylococcal disease. We know, too, that even if we limit ourselves to the staphylococci that regularly produce the *aureus* pigment and coagulase, or even to the members of the same bacteriophage type, we are not necessarily on firm ground with respect to their homogeneity in terms of ability to initiate disease in man or some laboratory animal. At times it seems as if every *Staphylococcus* is different.

Difficult Diagnoses

In addition to the strong individualistic tradition among staphylococci, consideration of the subject of staphylococcal infections or disease is further complicated by the very real difficulties in establishing a diagnosis. Here, we have the same general problem that we face with any ubiquitous microbe, but with the added obstacle of the knowledge that staphylococci are capable of producing lesions virtually anywhere on the body surface and that human carriers of staphylococci are commonplace. As a consequence we cannot accept the mere presence of staphylococci in an open lesion such as a wound, even an operative wound, with any degree of confidence that the microbe and the lesion are necessarily related. In short, open wounds on open wards, no matter how carefully the wound may be protected by surgical dressing, are seldom suitable objects for the study of staphylococcal infection and disease in spite of the fact that we know that some of these lesions *could* be related to the staphylococci.

Accordingly, in considering staphylococcal infection and disease, one must limit the discussion to lesions that had never shown external drainage at the time of first culture, that is, the so-called "closed lesions" and the infections accompanied by bacteremia. Even with the so-called "closed lesions" the chance of contaminating the lesion by an irrelevant *Staphylococcus* is present, but can be minimized with proper care.

These points are quite well known to all of us. Nevertheless, I am convinced

that we who are familiar with them have almost completely failed to communicate them to our scientific colleagues not directly connected with the staphylococcal field. We have sinned further, in my opinion, by not clearly pointing out that most of the large-scale clinical investigations on drug-resistant staphylococci were not concerned with staphylococcal disease at all, but had to do with something quite different: namely, the healthy carriers of staphylococci. Finally, while I am on the subject of our sins, until recently we quite failed to emphasize something we all knew, namely, that a penicillin-susceptible staphylococcal infection in a patient virtually never becomes penicillin-resistant during therapy. As a result of this failure in ordinary communication I am quite confident that physicians and others interested but not directly engaged in the field have the misconception that the number of patents with actual drug-resistant staphylococcal infections and disease seen each year on general hospital service can be numbered in the hundreds. There exists the further misconception that if one treats a penicillin-susceptible instance of serious staphylococcal disease with penicillin, the drug will soon prove valueless because of the emergence to predominance of drug-resistant staphylococci.

Recent Developments

With full appreciation of the problems presented by this individualistic tradition of staphylococci and the difficulties in diagnosis, let us turn now to the question of the staphylococcal problem as we face it today. Let us also try to see whether the problem is significantly different from that presented a decade or so ago.

First, let us ask whether our population at large or the special population in our hospitals is experiencing any greater exposure to staphylococci than was the case 10 or 12 years ago. Fortunately, careful studies bearing on this point are available and indicate that this is not the case. We have, for example, the detailed studies of A. A. Miles and his associates[1] in England, published in 1944, and we have many studies with the phage-typing method conducted in widely separated portions of the world during recent years. All of these studies show substantially the same results.

Approximately one half of any group of adults not in a hospital are nasal carriers of staphylococci when studied at a single point in time. When members of the group are followed individually for periods of several weeks or longer, the percentage harboring staphylococci at one time or another rises and may reach 70 or 80 per cent. Likewise, when patients are studied on first admission to a hospital and then throughout a hospital stay, the percentage harboring staphylococci goes up appreciably from the 50-per cent level on admission and then tends to fall back again after discharge.

One of the many interesting observations brought out in this type of study has been the statement of Miles, who said in 1944 that "The nasal carrier state varies, not with the environment of the person, but with the person himself. There is a marked tendency for persons to be either persistent *carriers** or persistently *free* from nasal *Staph. aureus*."

* Italicized by Walsh McDermott.

Included in this monograph is a paper by Vernon Knight showing that this rather fascinating phenomenon is also true today.

It appears, then, that in answer to the first question there is no indication that our degree of exposure to staphylococci either in or out of our hospitals is any greater today than it was 12 years ago. This is not particularly surprising, and it is perhaps just another way of saying that staphylococci were plentiful then and are plentiful now.

The next question that arises is whether there is any reason to believe that strains of staphylococci of enhanced pathogenicity for man are more prevalent today than they were 10 or 15 years ago.

This question is less readily answered than the previous question because of the nature of the problem. If such an increased prevalence of more pathogenic staphylococci were occurring, however, it would be most readily apparent in the number and the clinical forms of the staphylococcal disease seen in patients on admission to the hospital.

Infections occurring in the hospital environment itself would not provide much information on this subject unless the infections were truly overwhelming. The reason for this is that in a hospital population the presence of so many people ill with other diseases, or experiencing illness as the immediate after-effect of treatment, would make it difficult to distinguish between increased pathogenicity of the staphylococci or decreased resistance of the host in any individual situation.

In recent years there appears to have been surprisingly little attention directed toward patients newly admitted to hospitals. Everyone seems to agree that one of the commonest forms of serious staphylococcal disease, the primary hematogenous osteomyelitis of childhood and adolescence, has virtually disappeared from our admitting rooms. I also suspect that patients with carbuncles and recurrent boils are requesting admission to the hospital less frequently than was the case 10 or 15 years ago, but I cannot substantiate this suspicion. In several of the recent reports, for example one made by Barber and Burton[2] from London, the number and the form of staphylococcal disease seen in patients on admission to the hospital are listed, but no comment is made as to how the material compares with the experience in years past.

In the 20-year period from 1933 to 1953 at the Johns Hopkins Medical Center, Baltimore, Md., Fisher *et al.*[3] reported that slightly over 100 patients with staphylococcemia were seen. This number presumably also includes patients who developed bacteremia while in the hospital. Nevertheless, the crude figure of 5 to 10 patients admitted annually with staphylococcal bacteremia would be consistent with what has been seen at New York Hospital-Cornell Medical Center, New York, N. Y. It should be mentioned, moreover, that admission rates of patients with staphylococcal bacteremia to the large university centers may give a falsely high value because of the tendency to transfer such patients to the larger centers after therapeutic failure in a smaller hospital.

Another serious form of staphylococcal disease, the primary pneumonia of adults, tends to occur in sporadic outbreaks in association with viral influenza. It is difficult to form an estimate of the general annual incidence of this disease. It seems safe, however, to say that there has been no recent steady increase in

primary staphylococcal pneumonia in nonhospitalized patients. Such an increase, related or unrelated to influenza outbreaks, might be expected if strains of appreciably enhanced pathogenicity for man were becoming more prevalent.

There is 1 entity, the staphylococcal empyema of infants, particularly of those under the age of 6 months, that has been reported to be on the increase. This entity probably represents a situation that is closer to that of the development of infection in the *hospitalized* patient than to that of the adult *coming* to the hospital with established staphylococcal disease. If, accordingly, there is an increase in this entity, and the fact that a real increase has occurred is by no means clear, the increase should be considered in relation to the problem of the development of staphylococcal disease by persons with hospital experience.

In spite of this 1 possible reported exception, however, there is remarkably little evidence to suggest any notable increase in the number of patients, particularly adult patients, appearing at our hospital admission rooms with staphylococcal disease. Moreover, there is nothing to suggest that the forms of the staphylococcal disease seen might represent the dissemination of more malignant strains of the microbe or the concentration of more malignant strains in particular localities outside of our hospital. Indeed, the one apparently definite change in the form of observable staphylococcal disease is for the better, namely, the virtual disappearance of the serious diseases of primary hematogenous osteomyelitis.

The evidence available on the form and numbers of patients with staphylococcal disease requesting hospital admission is admittedly sketchy. Nevertheless, much has been published on staphylococci in recent years. Yet, no one seems to be mentioning either any remarkable increase in the number of patients with staphylococcal disease or any increase in unusually malignant forms among the patients who present themselves for admission to the hospital. We have no reason for believing that there has been any spread of unusually virulent staphylococci among what might be termed "the public at large."

We have, therefore, a situation in which neither our patients in hospitals nor the public at large are apparently undergoing any greater exposure to staphylococci than was the case a decade or so ago. Also, we have no particular reason to believe that there has been the wide increase in the prevalence of unusually pathogenic staphylococci, or even that there has been any substantial numerical increase in staphylococcal disease occurring outside our hospitals.

In brief, we have no convincing evidence or, indeed, even any particularly well-founded suspicion, *that the crude equilibrium between the public at large and the ubiquitous staphylococci is shifting for the worse.* If any shifts have occurred they seem to have been on the side of a more favorable situation for the members of the public.

Staphylococci in Hospitals

What, then, is the situation of our special communities of hospitalized patients with respect to their crude equilibrium with their staphylococci?

As mentioned before, their risk of exposure to staphylococci *in general* is presumably no different than it ever was, or at least no different than it has been in the past decade. There is the possibility, however, that there is now a wider prevalence of particular strains of increased pathogenicity for man. Because of the localized and highly special circumstances involved, is it not possible that especially pathogenic strains of staphylococci might be lurking in the hospital environment, whereas the same strains would not be so readily detectable when spread through the community at large?

Let us set a measure of increased virulence in terms of epidemics of unusually serious staphylococcal disease involving a dozen or more patients at a time in a single hospital and occurring throughout our hospitals in general. Viewed in these terms I do not believe that we have a particularly good case for increased virulence of the so-called "hospital staphylococci." When one considers the highly individualistic nature of staphylococci, however, it is by no means inconceivable that such strains are present here and there, though operating, thus far, only in terms of tiny localized epidemics. Indeed, there is some reason to believe, in the case of a few strains, notably the enterotoxin-producing strains, that this sort of thing is actually happening even though its over-all numerical expression is still quite small.

Even this occurrence of tiny but multiple epidemics involving enterotoxin-producing staphylococci, however, does *not* establish that these strains are necessarily newcomers to our hospitals or that they have necessarily *assumed increased virulence for man*. We are obviously doing other things to our patients that we were not doing in years gone by, and it may be that *it is the setting rather than any absolute increase in microbial virulence* that determines this alarming phenomenon.

There is, however, a phenomenon recently reemphasized by Barber and Burston[2] that might indicate that the staphylococci prevalent in our hospitals today have an increased pathogenicity for man. In brief, these workers have presented evidence that strains of staphylococci from the lesions of active staphylococcal disease may be the chief offenders in terms of cross-infection and in the ability to initiate staphylococcal disease in new hosts. It should be emphasized that these investigators do not present their findings as evidence that the lesion-producing staphylococci are necessarily more virulent than the more virulent of our present strains. They merely suggest that it is these lesion-producing staphylococci that have the capacity to initiate new disease in relative contrast to the staphylococci of the nasal carriers.

These observations made by Barber and Burston, who used modern techniques, are particularly timely and should form the basis for detailed epidemiologic investigations. It is no disparagement to point out that the findings recall to mind the well-respected item in the clinical lore of our fathers' generation to the effect that a hand infection in a surgeon or a pathologist was a far more dangerous thing than a hand infection in a housewife who had stuck herself with a pin. It was reasoned that the strain of microbe in the case of the surgeon or the pathologist was more dangerous on the grounds that it had been so recently producing disease in a patient.

Although these last few remarks on hospital staphylococci are relevant to the

central topic, they represent a slight digression from the consideration of whether today's hospital staphylococci contain an impressively increased prevalence of unprecedentedly virulent strains. On this subject I believe that, when one considers the wide range among individual strains of staphylococci in terms of ability to initiate new human disease, there is no convincing evidence that our hospital staphylococci, on the whole, are any more virulent for man than they ever were.

As I see it, therefore, we are not subjecting our patients in hospitals to a greater risk of exposure to staphylococci or to any more especially virulent strains of staphylococci than we have ever done.

This does not mean that we are not seeing the development of more staphylococcal disease among our patients while they reside in our hospitals today than was the case a decade or so ago. Indeed, the only reason for qualifying the situation at all is the lack of precise comparative data for the 2 time periods. Despite the absence of precise data, I am confident that most of us are convinced that today we are seeing, with greater frequency, the actual development of staphylococcal disease in patients *after* they have come into our hospitals. In short, we are beginning to detect signs that the hitherto satisfactory equilibrium between our hospital patients and staphylococci is becoming unbalanced.

It is not so much that our patients in hospitals are being subjected to any greater challenge by staphylococci as it is that they are less well equipped to meet the same old challenge. Because they are less well equipped, it is necessary that they receive better protection. At the present time, however, we are doing considerably less than offering this protection. Indeed, in many cases we are actually lowering those few defenses the patient brings with him to the hospital. We are doing this with the bodily disturbances we create as an unsought-for accompaniment of many of our modern therapies. In effect, an unfortunate by-product of our life-saving and life-prolonging procedures is the creation of a favorable breeding place for staphylococci.

This favorable breeding place is being created in several ways. First, our modern therapies are permitting the survival of people who are less able, in various undefined ways, to cope with bacterial infections. Examples of this sort are the insulin-treated diabetics, the children who have had splenectomies for various hematologic disorders, the adults who have had total gastrectomies, and perhaps even some who have had partial pulmonary resections. Second, certain of our commonly used treatments such as cortisone, hydrocortisone and corticotropin, the more widely acting antimicrobials, and X-ray irradiation are known to create circumstances that, at times, facilitate the development of infection. It is conceivable that on certain occasions treatments such as the antihistaminics or the anticoagulants might unfavorably influence infection. Finally, in order to manage many of these modern treatments properly, it is necessary to perform venipunctures and skin punctures with unusual frequency on these very patients who, for one reason or another, are more liable to the development of infection.

To be sure, this increased congregation of more susceptible hosts in our hospitals is not necessarily the only reason for the apparent increase in staphylo-

coccal infections developing in the hospital. Nevertheless, it does seem to represent the principal difference between our situation today and that of a decade or so ago. Moreover, if this increase is occurring in the hospitals today, it is reasonable to expect that it will occur outside the hospital in the future as these various forces become operative on a wider scale.

Assuming the validity of this analysis, how can the situation be improved? It would be pointless to think in terms of attempting to oppose many of the forces that are contributing to this situation because so many of these forces are such notable scientific achievements for the common good. Moreover, as staphylococci are ubiquitous, an approach based on attempts to eliminate them from the environment would not seem hopeful. In the same way, it does not appear that antimicrobial drugs, per se, would play an important role. Chemoprophylaxis is, at best, a two-edged sword. In the present situation the necessity for protecting the patient for such very long periods against a number of microbial species in addition to staphylococci makes the use of drugs impracticable.

Two approaches exist that seem reasonable. The first is for us all to have a continuing awareness of the fact that our hospitalized patients today are, on the whole, less able to cope with staphylococci than was formerly the case. This means that we should review and probably intensify our aseptic practices, not only in the operating room, but especially in connection with the ordinary "puncture" procedures used in the wards.

The second approach is the major one, namely, to find means for increasing bodily defenses against staphylococci and to find means for restoring these defenses when they have been reduced. The logic of this approach is evident if you agree with me that the crux of today's staphylococcal problem lies not so much in changes in our staphylococci as it does in changes in the status of their hosts. The difficulties with this second type of approach arise immediately, however, and stem from the fact that we are almost wholly ignorant of the mechanics of staphylococcal infection and disease. Until we can discover more than we know now concerning what determines the pathogenicity of certain staphylococci and how the healthy host manages to live in peace with them, it is not likely that our situation will materially improve.

Therefore, this monograph, which is so largely dedicated to this central theme, represents a most timely and important study for all of us who are trying to learn more about staphylococcal disease.

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Discussion of the Paper

E. T. Bynoe (*Bacteriological Laboratories, Laboratory of Hygiene, Department of National Health and Welfare, Ottawa, Ont., Canada*): In reviewing the

staphylococcal problem as it exists today inside the hospital and in the community at large, as Walsh McDermott states, ". . . we should review and probably intensify our aseptic practices, not only in the operating room but especially in connection with the ordinary 'puncture' procedures used in the wards." In Canada, a special committee has been studying the problem of staphylococcal infections in our D. V. A. hospitals for the last couple of years, and we are agreed that the strictest attention to good aseptic techniques offers the best, perhaps the only, hope of controlling staphylococcal cross-infection in the hospital. It is generally admitted that during the past 15 years techniques have deteriorated because antibiotics have been increasingly relied upon to take care of any contamination that might occur. With the widespread prevalence of resistant staphylococci in our hospitals today, we can no longer rely on these drugs to prevent staphylococcal cross-infection, and we are forced to go back and take a good, critical second look at all our hospital practices with a view to tightening our defenses so as to block the transmission of these ubiquitous pathogens within the hospital. The sources, reservoirs, and means of transmission of staphylococci in the hospital are so numerous that the prevention of hospital-acquired infection is a particularly difficult problem. Dust suppression, proper dressing techniques, isolation, chemical treatment of bedding, adequate ventilation, removal of carriers, occlusive dressings, and other means have all been demonstrated on occasion to lower the incidence of hospital-acquired infections. It is only, however, by trying to control all the means whereby the staphylococci are spread that we can expect to reduce to a minimum infections acquired in the hospital. It requires the concerted interest and action of all hospital personnel—surgical, medical, nursing, and housekeeping staffs. This is the plea we are making today, and the approach we are stressing is a return to and an improvement upon Listerian principles of aseptic and antiseptic techniques in our hospitals.

STUDIES ON THE FATE OF VIRULENT AND AVIRULENT STAPHYLOCOCCI IN MICE*

By J. Maclean Smith

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An experimental intravenous infection in mice has been used to study the behavior of different strains of staphylococci.[1] The method of Fenner, Martin, and Pierce[2] and Pierce, Dubos, and Schaefer was used to quantitate the survival of staphylococci in mouse tissues.[3] FIGURE 1 shows schematically the method in which these experiments were performed. The organism to be tested was grown in peptone broth overnight at a temperature of 37° C. The mice used were 3 to 4 weeks old and 0.1 cc. of culture was administered intravenously via the tail veins. The animals were restrained in small glass containers with the tails projecting as shown in the figure. At varying intervals after innoculation the animals were killed, and the organs were removed with sterile precautions and placed in tubes for homogenization. The grinding was carried out in Pyrex tubes with plastic grinders (see illustration in Pierce *et al.*[3]). By this means the organs were ground to a homogenate in a few seconds. A small quantity of this suspension was diluted tenfold in tubes containing either 0.1 per cent albumin solution or tap water. From each dilution tube a small specimen contained in a platinum loop was removed and plated on a nutrient agar plate. These plates were incubated overnight at 37° C. On the following day, counts were made from dilutions containing less than 200 colonies. From the information thus obtained the number of organisms present in the original organ was calculated.

A series of experiments was carried out with the organisms *Staphylococcus* "Smith." This organism was originally isolated from a patient with osteomyelitis. It has been derived from the same source as SA 235. Details of such an experiment are shown in FIGURE 2. It will be seen that the organisms were initially plentiful in the spleen, but over a 2-week period they gradually decreased so that on the 14th day, *by this technique*, we were unable to detect any organisms. The same was true of the organisms in the liver. In the lungs, however, a different situation obtained. Here the initial number of organisms was lower but staphylococci tended to persist throughout the experimental period. The organisms initially present in the kidney were small in number but progressive multiplication occurred. At the time of death, occurring between the 7th and the 14th day, the staphylococci in the kidney were at a peak titer. The surviving animals showed a fall in titer from this peak level. FIGURE 2 is representative of the results of a number of experiments of the same type. Further experiments were done, in which the animals were killed 1 to 2 minutes after innoculation. It was found that the initial titer of organisms in all the organs was higher at this time, and that a marked drop in the

* The first part of this investigation was carried out in cooperation with R. J. Dubos in his laboratories at the Rockefeller Institute for Medical Research, New York, N. Y. The second part was carried out in the Infectious Disease Laboratory, Department of Internal Medicine, The State University of Iowa, Iowa City, Iowa, and was supported by research grant No. R.G. 4532 from the National Institutes of Health, Public Health Service, Department of Health, Education, and Welfare, Bethesda, Md. The author acknowledges the valuable technical assistance of Joan A. Becker and Phyllis D. Beals.

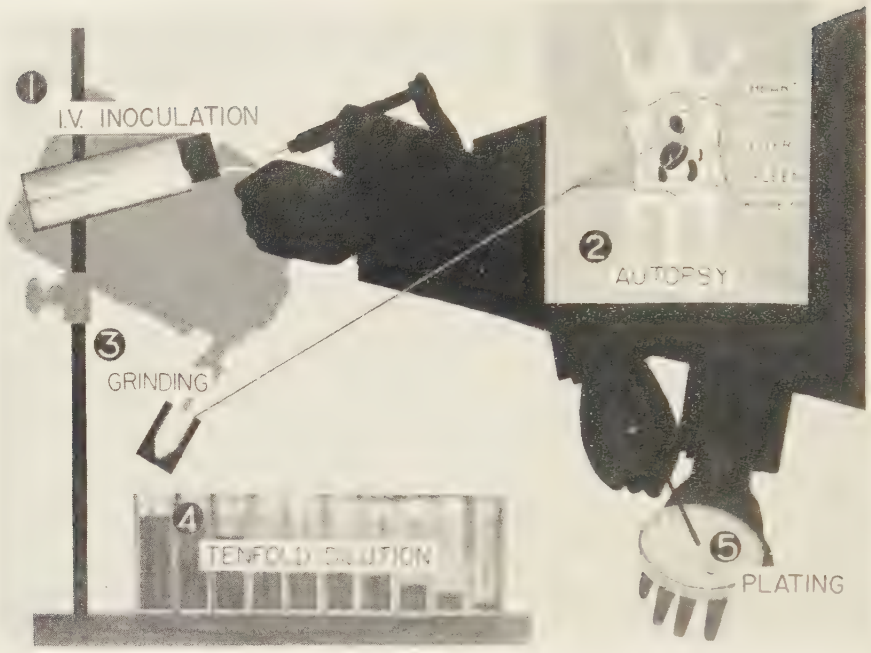

FIGURE 1. The method used in enumerating staphylococci in mouse tissues

surviving number of staphylococci occurred during the first hour. Other experiments have also shown that organisms are present in the blood for the first 24 hours and occasionally slightly longer.

A study was made of a number of organisms, some coagulase-positive and others coagulase-negative. In general the multiplication of different staphylococcal strains was similar except within the kidney. FIGURE 3 shows the fate of the organisms in the kidney in a number of animals studied over a period of time. Each point on these graphs represents the mean titer of kidney staphylococcal populations of 4 to 12 animals. The top 3 lines represent animals infected with coagulase-positive organisms, namely "Giorgio," a coagulase-positive *Staphylococcus aureus* organism isolated from an infected craniotomy wound, "Smith," the strain already referred to, and "O'Hara," which is *aureus* coagulase-positive and was isolated from a case of bronchio pneumonia. The 3 bottom strains, "Air," "Stern," and "J.A.B.," are all coagulase-negative *Staphylococcus albus* organisms isolated, in the case of Stern and J.A.B., from normal skin, and from the air in the other case. The seventh organism on this chart, M.A.M., is a *Staphylococcus albus* that was initially coagulase-negative but appeared to change its characteristics in the laboratory and is at present weakly coagulase-positive. It is evident that there was a difference in the growth of these organisms in the mouse kidney. The coagulase-positive organisms grow better in the kidney than do the coagulase-negative ones. There is a further difference within these 2 groups, however. Some coagulase-positive

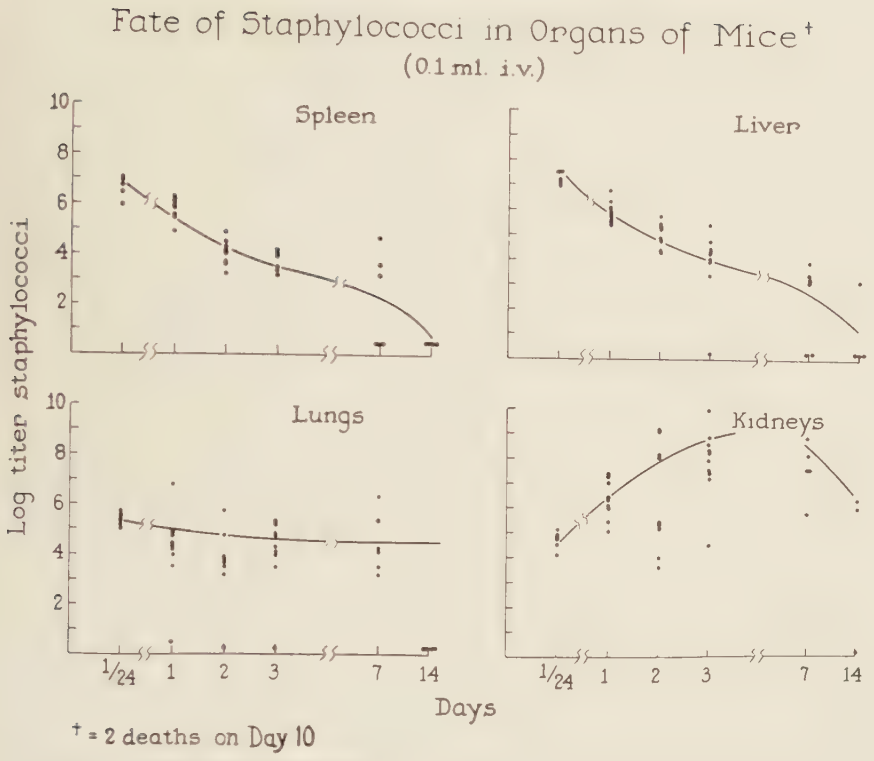

FIGURE 2. The distribution of staphylococci in the organs of mice throughout a 2-week period. The lines in this figure are trend lines.

organisms grow better than others. The same thing is true of the coagulase-negative group. The M.A.M. organisms with intermediate coagulase activity are also intermediate in their growth in the kidney. Comparison of this study with others involving other virulent-avirulent pairs of organisms allows us to make a hypothesis: Avirulent organisms differ from their virulent counterparts in their ability to grow in a "target" organ.

Organisms collected from patients in the University Hospitals, Iowa City,* Iowa, have been tested in mice for their lethal effect and for their growth in the kidney. These organisms were collected from the skin, sputum, or lung, blood, throat, bone, and ear. They were grown in a way similar to that used in the last experiment and were also injected in 0.1 cc. volume of culture. In each case 15 4-week-old mice were injected, 10 for mortality studies and 5 for the kidney titer. FIGURE 4 shows the effects seen with these different organisms. It is at once evident that although this dose is suitable for laboratory cultures it appears to be too high for the cultures newly isolated from patients. In a large number of cases all 10 animals injected

* Kindly collected by members of the Department of Bacteriology, State University of Iowa, Iowa City, Iowa, from routine cultures.

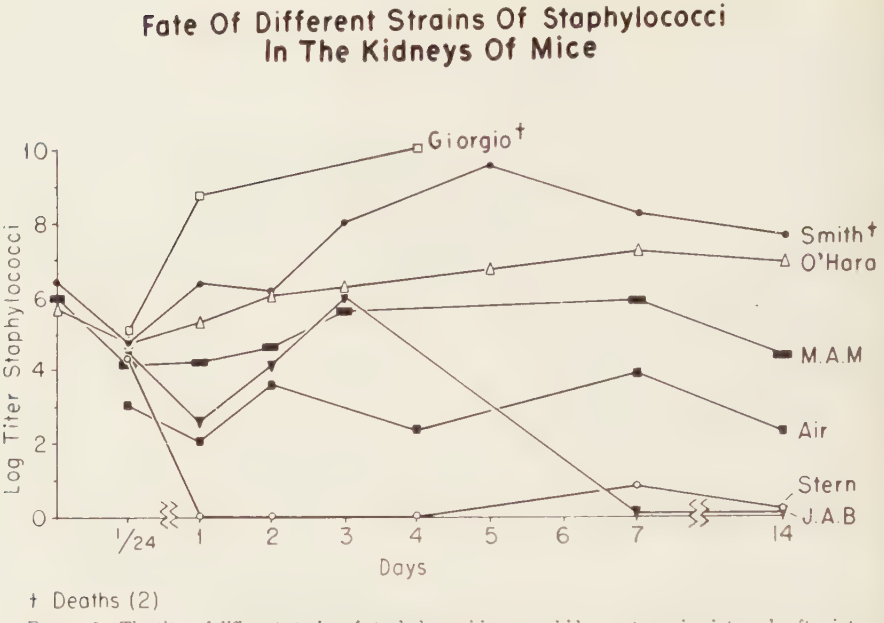

FIGURE 3. The titer of different strains of staphylococci in mouse kidneys at varying intervals after intravenous inoculation.

for mortality studies died. The organisms isolated from osteomyelitis cases did not kill as many mice as did organisms from other sources. It is of interest in this connection that, in surveys of human cases of septicemia, the death rate in cases following osteomyelitis was lower than the death rate in cases arising from other foci. Due to the high mortality we have rather few survivors in which to test the kidney titer. Those that we have been able to test are shown in the bottom section of FIGURE 4. It is possible that this test may be useful in differentiating types of staphylococci. In its present form, however, this test is not as useful as it may be in the future.

A review of staphylococcal septicemia in patients at the University Hospitals is at present being carried out,[1] and we have analyzed the results with the first 100 cases. Forty-one of these cases died of the infection and, in this group, 22 autopsies were carried out. In the autopsy group the lungs and kidneys were most often affected. In women, respiratory disease was commonest and, in men, kidney disease was most frequent. In the whole autopsy group the kidneys were grossly involved in 68 per cent of cases. In an attempt to investigate the possible presence of renal failure and its contribution to death in these cases, creatinines, when available, were examined for the group of 100 cases. In 12 surviving cases, 2, or 17 per cent, were abnormal whereas, in 25 fatal cases, 10, or 40 per cent, were abnormal. Similarly, the blood urea was abnormal in 20 per cent of the surviving cases and in 61 per cent of the fatal cases. We therefore feel that it is probable that kidney malfunction contributes to death, and that the ability of the staphylococci to grow in the

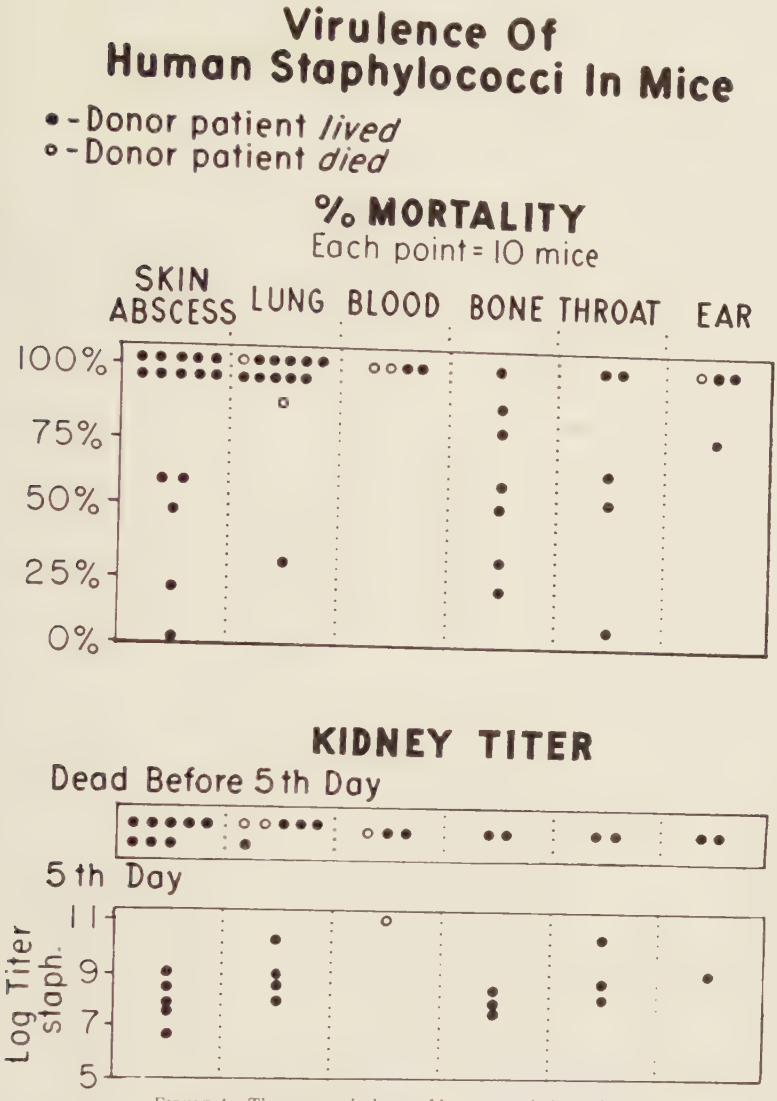

FIGURE 4. The mouse virulence of human staphylococci.

human kidney may well be a contributing factor to the virulence of staphylococci in human patients.

Summary

An experimental staphylococcal infection in mice has shown that the organisms survive best in the lungs and kidneys. A comparison of strains has shown a spectrum of virulence in mice related to the ability of the organisms to multiply in the mouse kidney. Organisms isolated from patients with

staphylococcal disease have been studied from the point of view of their virulence in mice. A study of human staphylococcal septicemia indicates that renal disease is an important factor and that it may be a contributory factor in causing death in fatal cases.

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THE BLOOD STREAM CLEARANCE OF STAPHYLOCOCCI IN RABBITS*

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Numerous experimental studies have demonstrated that most species of microorganisms are rapidly removed from the peripheral blood following their intravenous administration.[2-6, 15] Although considerable data are available regarding the mechanisms responsible for the initial clearance of microorganisms from the blood stream, little attention has been directed toward the low level of bacteremia that may subsequently persist for many hours.

Previous studies made in this laboratory have shown that staphylococci may survive for long periods of time within the cytoplasm of leukocytes.[10] The present investigation was directed toward host factors that might permit small numbers of staphylococci to persist within the circulating blood of rabbits. These studies suggest that coagulase-positive staphylococci are swiftly incorporated within circulating leukocytes and that such intraleukocytic residence may prevent their effective removal from the circulation.

Material and Methods

Preparation of rabbits. Healthy male rabbits of mixed stock weighing from 2.8 to 3.8 kg. were used in these experiments. In the performance of clearance studies both jugular veins were exposed through a thyroid incision, and catheters were passed into the superior vena cava and the left hepatic vein under fluoroscopic control. Both catheters were then led out subcutaneously through a small dorsal incision in the skin of the back, and the thyroid incision was closed. The animals were allowed to recover from anesthesia before clearance studies were initiated, and they stood unrestrained during the withdrawal of blood specimens.

Procurement and plating of blood specimens. Microorganisms were injected via the marginal ear vein in 2.0 ml. of normal saline. At each sampling period both catheters were cleared by the removal of 2.0 ml. of blood, and samples for study were simultaneously obtained from both catheters. The blood was delivered to sterile tubes containing sufficient dried heparin to make a final concentration of 1:10,000. These tubes were maintained at 4° C. in an ice-salt bath. All specimens were appropriately diluted in tenfold distilled water blanks at 4° C. for duplicate platings within 5 minutes after withdrawal from the animal.

Determination of intravascular phagocytosis. To detect the intravascular phagocytosis of microorganisms, a centrifugation technique described by Maaløe[7] was used. Preliminary control experiments were performed to determine

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a centrifugation procedure that would sediment all leukocytes but would not affect extracellular microorganisms. Repeated control studies indicated that the number of microorganisms remaining in the supernatant following such centrifugation was not altered by incubation in plasma or in chilled whole blood by the presence of red cells or injured leukocytes. The degree of incorporation of microorganisms within leukocytes was then calculated from the differences in plate counts obtained from each total blood specimen, and the plate counts obtained from the supernatant following standard centrifugation.

Cultures. A strain of *Staphylococcus* designated MAM was used in all these clearance studies. An 18-hour peptone infusion broth culture was used as the inoculum. This organism was a coagulase-positive *staphylococcus* that fermented mannite, was phosphatase-positive, and was lysed by phage 52A (for further description of the characteristics of this strain see Smith and Dubos[11]). A type-III pneumococcus originally obtained from Colin M. MacLeod was also used in some clearance experiments.

Experimental

The clearance of large numbers of staphylococci from superior caval blood. When rabbits were given 5×10^8 viable staphylococci intravenously, a consistent and reproducible clearance curve was obtained. Approximately 1 million culturable staphylococci were present in superior caval blood 1 minute following injection. During the succeeding 20 minutes a swift 1 thousand-fold reduction in the circulating microbial population occurred. At 15 to 20

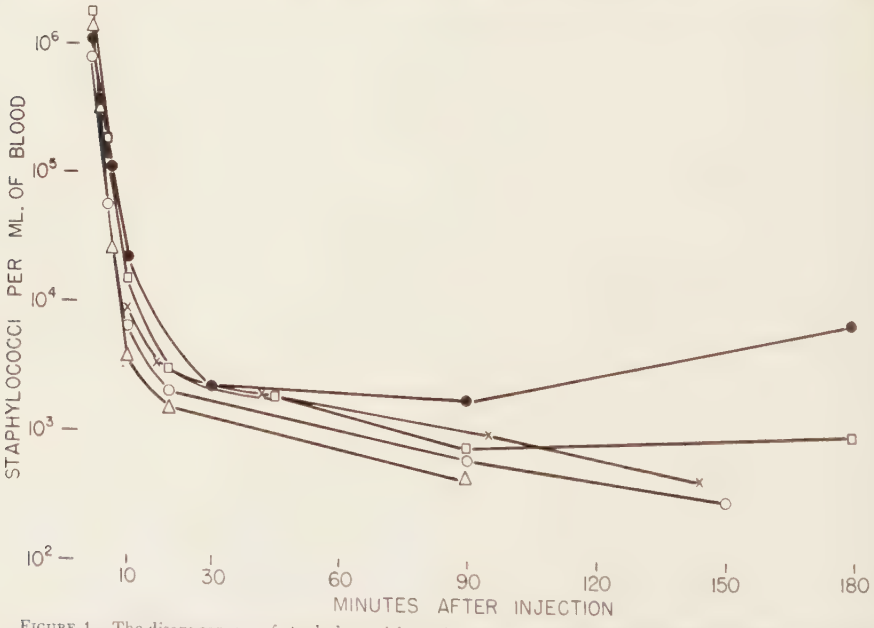

FIGURE 1. The disappearance of staphylococci from the blood stream of normal rabbits following the intravenous injection of 5×10^8 viable units. Bacteremia of 1,000 to 2,000 staphylococci per ml. persists following the initial rapid clearance phase.

minutes, however, there was an abrupt reduction in the rate of clearance, resulting in a bacteremia of 1,000 to 2,000 staphylococci per ml. which persisted for long periods of time. Typical results obtained in 5 normal rabbits are illustrated in FIGURE 1.

When the sampling period was extended beyond the 180 minutes noted in FIGURE 1, a slow decline in the level of bacteremia ensued. Nevertheless, the superior cava was never completely cleared of microorganisms, and a secondary increase in the level of bacteremia occurred 3 to 15 hours after the injection of culture.

The trapping of circulating staphylococci within the splanchnic viscera. When simultaneous hepatic vein cultures were studied, a definite pattern of splanchnic

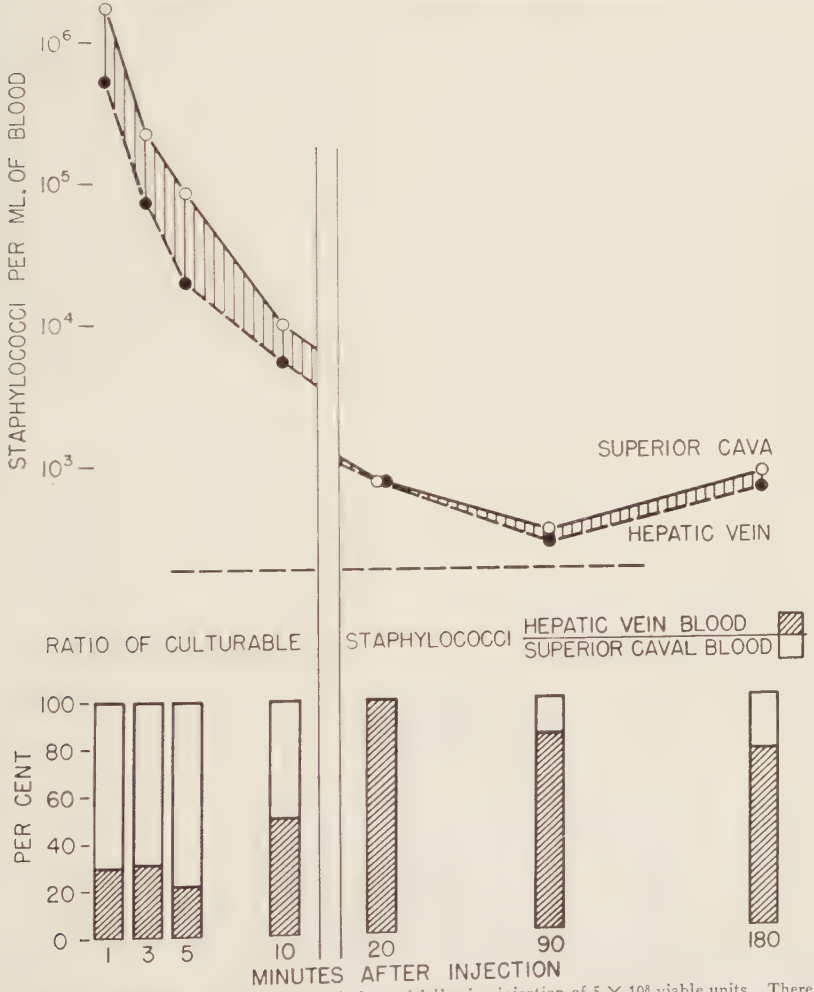

FIGURE 2. The splanchnic removal of staphylococci following injection of 5×10^8 viable units. There is a progressive decrease in the number of circulating staphylococci sequestered in transit through the splanchnic viscera. Hepatic and superior caval blood contain equal numbers of culturable staphylococci at 20 minutes.

trapping of staphylococci became apparent. Hepatic blood specimens obtained during the rapid phase of clearance at 1, 3, and 5 minutes revealed only 20 to 25 per cent as many staphylococci as cultures of superior caval blood (FIGURE 2).

These findings were consistent from experiment to experiment, and they appeared to indicate efficient splanchnic trapping of staphylococci during this initial period. A progressive reduction in the degree of splanchnic removal then ensued. Within 20 minutes the number of culturable staphylococci in hepatic and superior caval blood was essentially equal, and little evidence of splanchnic trapping was apparent at subsequent sampling intervals.

Such a decline in the degree of splanchnic removal of staphylococci suggested that saturation of removal mechanisms might account for the persistence of bacteremia. Experiments were thus performed in which a second intravenous injection of staphylococci was given when splanchnic trapping had significantly declined or virtually ceased. Such an experiment is illustrated in FIGURE 3.

As noted in FIGURE 3, 75 to 80 per cent of the circulating staphylococci were removed in the splanchnic bed during the first 10 minutes after the injection of 5×10^8 staphylococci. Ninety minutes after the initial injection, splanchnic removal had significantly declined, only 35 per cent of the circulating microorganisms failing to traverse the splanchnic viscera. A second injection of 5×10^8 staphylococci at this time resulted in rapid clearance which paralleled that clearance obtained following the initial injection of culture. Again hepatic blood cultures obtained during the second rapid phase of clearance revealed evidence of efficient splanchnic trapping. Ninety minutes following

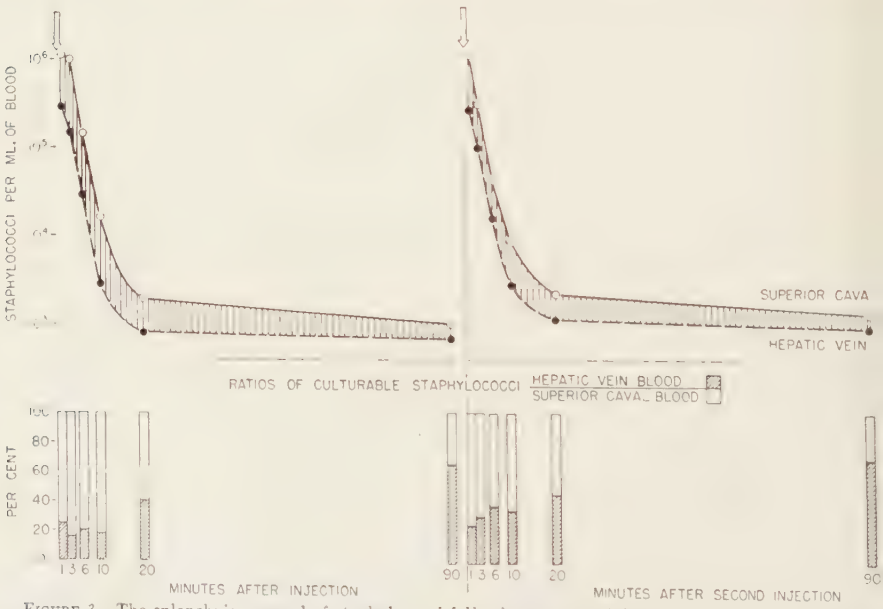

FIGURE 3. The splanchnic removal of staphylococci following 2 spaced injections of 5×10^8 viable units. Despite a progressive reduction in splanchnic trapping following the initial injection of culture, splanchnic removal is again efficient following a second identical inoculum.

the second injection, however, superior caval-hepatic vein differences had again declined significantly, and only 30 per cent of the circulating staphylococci appeared to remain in the splanchnic viscera. Such experiments appeared to rule out simple saturation of the splanchnic removal system as an explanation for the persistent bacteremia.

Experiments in which the injected inoculum was reduced 1 thousandfold indicated that low initial levels of circulating staphylococci per se did not change initial clearance rates or reduce the degree of splanchnic removal of staphylococci.

The intravascular phagocytosis of staphylococci. Review of the data obtained in these experiments suggested that intravascular phagocytosis might account for the phenomena observed. When blood specimens obtained during clearance were differentially cultured before and after the differential centrifugation procedure previously outlined, evidence of swift intravascular phagocytosis was obtained. FIGURE 4 represents a composite graph of the degree of intravascular phagocytosis noted in 6 experiments. As seen in FIGURE 4, intravascular phagocytosis appeared to take place extremely rapidly, with 90 per cent of the microorganisms apparently residing within leukocytes within 20 minutes. Throughout the remainder of the 180-minute clearance-period graph, virtually all of the viable circulating staphylococci appeared to be contained within leukocytes.

The transport of intracellular staphylococci through the splanchnic viscera. To determine whether intracellular or extracellular microorganisms were preferentially removed in transit through the liver and spleen, similar centrifugation

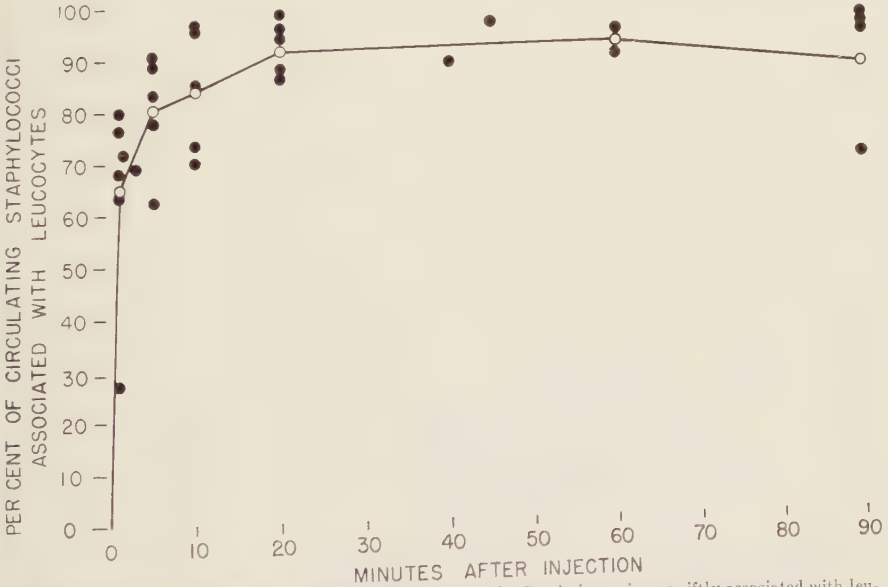

FIGURE 4. The intravascular phagocytosis of staphylococci. Staphylococci are swiftly associated with leukocytes. Virtually all of the circulating staphylococci appear to reside within cells 20 minutes after intravenous administration of the culture.

TABLE 1

THE PERCENTAGE OF STAPHYLOCOCCI CONTAINED WITHIN LEUKOCYTES IN SUPERIOR VENA CAVAL BLOOD AND HEPATIC VEIN BLOOD DURING BLOOD-STREAM CLEARANCE OF STAPHYLOCOCCI

Time after injection	Superior vena cava		Hepatic vein		Ratio Staphylococci in hepatic vein / Superior cava
	Staphylococci per ml. blood	Per cent within leukocytes	Staphylococci per ml. blood	Per cent within leukocytes	
1	2	3	4	5	6
1 min.	2,100,000	72	440,000	88	0.21
5 min.	115,000	89	27,000	92	0.24
10 min.	11,500	88	8,100	95	0.70
20 min.	4,000	96	2,750	94	0.69
90 min.	2,900	not done	3,200	not done	1.1
3 hrs.	5,100	76	1,750	>99	0.34
20 hrs.	11,100	not done	5,300	not done	0.48

studies were performed on both superior caval and hepatic vein blood during the course of clearance. Such an experiment is recorded in TABLE 1. Columns 3 and 5 indicate the percentage of microorgansism contained within leukocytes at each sampling period.

As noted in columns 3 and 5 of TABLE 1, intravascular phagocytosis occurred rapidly, with over 70 per cent of the culturable staphylococci in superior caval blood appearing within the cellular layer at 1 minute. In all samples except the 20-minute specimen, the percentage of staphylococci associated with leukocytes was somewhat higher in hepatic vein blood, suggesting that microorganisms within cells might preferentially traverse the splanchnic bed.

In this particular experiment an increasing bacteremia occurred during the period of study, superior caval counts rising from 2,900 microorganisms per ml. at 90 minutes to 5,100 microorganisms per ml. at 3 hours. Differential culture of the 3-hour superior caval specimen indicated that only 76 per cent of the circulating microorganisms were now contained within cells, suggesting the entrance of extracellular staphylococci into the circulation. Simultaneous hepatic blood samples obtained at 3 hours revealed that virtually all of the culturable staphylococci traversing the splanchnic bed resided within cells at this sampling interval. As noted in column 6, splanchnic removal of microorganisms had gradually declined during the course of the experiment. As more extracellular staphylococci appeared in the circulation at 3 hours, splanchnic trapping was again evident, only 34 per cent of the circulating staphylococci now appearing in hepatic blood. Such findings have suggested that, as extracellular staphylococci re-enter the blood stream during the period of increasing bacteremia, splanchnic removal mechanisms are once again effective, and that extracellular staphylococci are preferentially sequestered in transit through the splanchnic bed.

Alterations in clearance produced by changes in Staphylococcus-*leukocyte relationships.* Two additional series of experiments were performed to determine the effect of intraleukocytic residence on the clearance of staphylococci. In 1

series of experiments rabbits were made granulocytopenic by the injection of 1.75 mg. of nitrogen mustard per kg. of body weight. Daily white blood cell and differential counts were performed, and clearance observations were made when circulating granulocytes had been reduced to less than 25 polymorphonuclear leukocytes per cu. mm.

In a second group of rabbits, exudates of polymorphonuclear leukocytes were created by the intraperitoneal injection of saline containing small amounts of soluble starch. The living polymorphonuclear leukocytes were harvested and allowed to phagocyte staphylococci *in vitro*. Such phagocytic mixtures, containing many intracellular staphylococci, were then injected into the same animal from which the leukocytes had originally been harvested, and clearance followed by serial blood cultures. The results of such studies are recorded in FIGURE 5. Observations on 2 granulocytopenic rabbits, 2 rabbits receiving "intracellular" staphylococci, and 4 normal animals are summarized. Each of these animals received between 4 and 6 $\times$ 10^8 viable units as an intravenous inoculum.

As noted in FIGURE 5, initial clearance rates were similar. At 20 minutes and in subsequent specimens, however, striking differences in the levels of persisting bacteremia were apparent. Animals receiving "intracellular" staph-

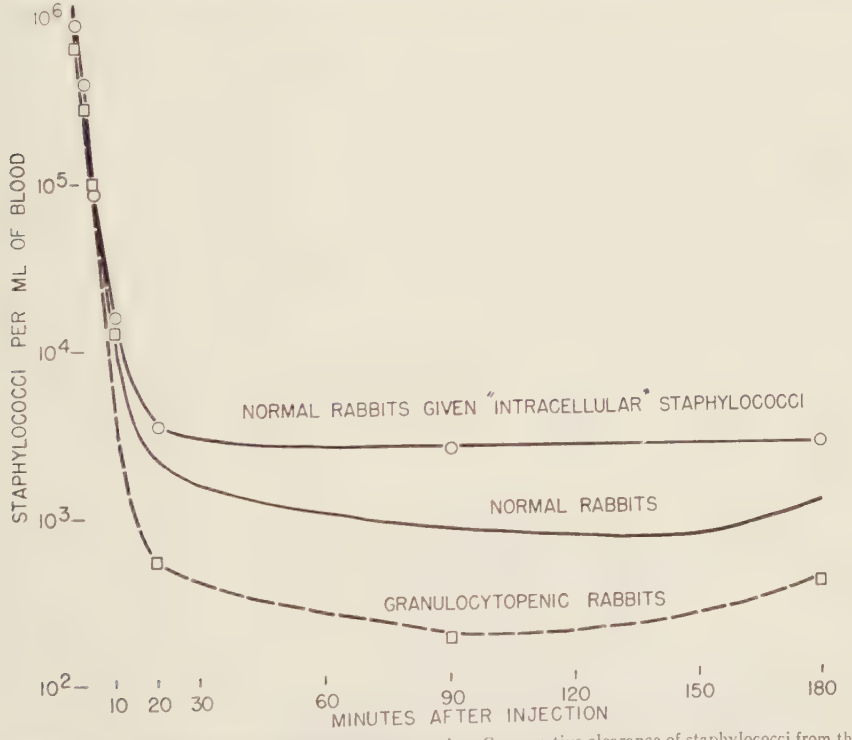

FIGURE 5. Changes in the level of persisting bacteremia. Comparative clearance of staphylococci from the blood stream of normal and granulocytopenic rabbits, and rabbits given "intracellular" staphylococci.

ylococci maintained a bacteremia that averaged 5 times that seen in normal animals. In contrast, granulocytopenic animals reduced the level of bacteremia to approximately one fifth that seen in normal animals. These observations tended to confirm the thesis that residence within circulating leukocytes protects staphylococci from removal from the blood stream in the reticuloendothelial system.

The removal of type-III pneumococci from superior caval blood. To determine whether intravascular phagocytosis occurred following the intravenous injection of other microorganisms, clearance studies were performed following the injection of a type-III pneumococcus. When similar numbers of pneumococci were injected intravenously, striking differences in the clearance of the microorganism from superior caval blood were noted. Initial levels of bacteremia were similar following the injection of either staphylococci or pneumococci. The number of pneumococci present in peripheral blood fell steadily at somewhat slower initial rates than during the clearance of staphylococci. No significant decline in the rate of clearance subsequently occurred, however, and pneumococci disappeared or fell to extremely low levels (less than 10 microorganisms per ml.) 45 to 90 minutes after the injection of culture. Studies on the degree of intravascular phagocytosis of viable pneumococci, carried out

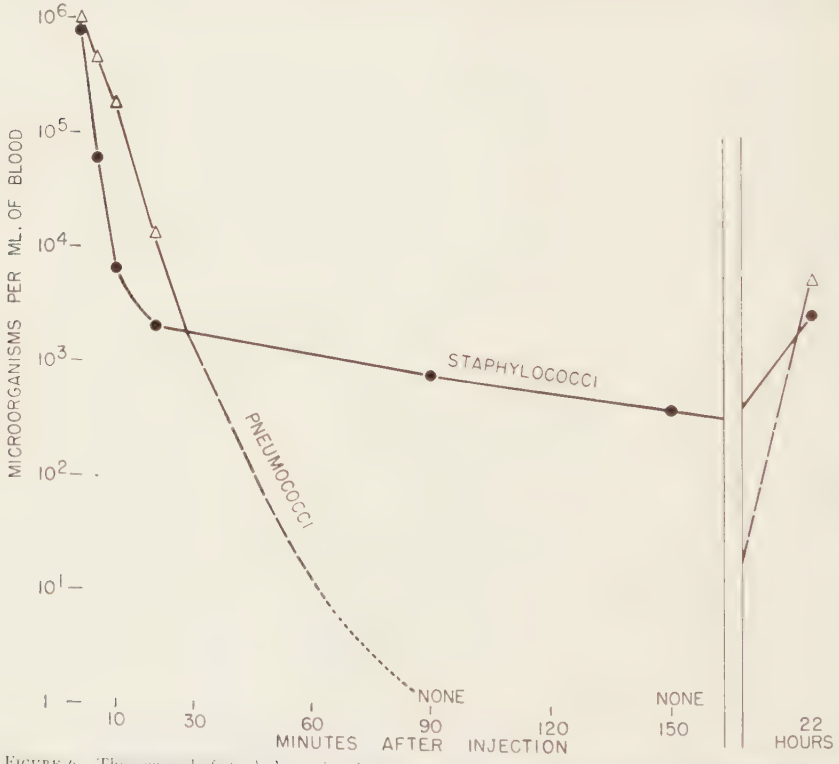

FIGURE 6. The removal of staphylococci and pneumococci from the blood stream of a single rabbit following simultaneous injection of both microorganisms.

with differential centrifugation, consistently indicated that all of the culturable pneumococci resided extracellularly in the plasma layer.

To check the significance of such differences in clearance further, 2 rabbits were simultaneously injected with both pneumococci and staphylococci, and blood samples were simultaneously plated in Todd-Hewitt agar and Todd-Hewitt agar containing 0.015 per cent oleic acid. Numerous preliminary studies demonstrated that oleic acid in these concentrations completely eliminated the growth of pneumococci but had no affect on the quantitative recovery of staphylococci. Such an experiment is graphically represented in FIGURE 6.

Staphylococci swiftly disappeared during the initial 10-minute period. Clearance then abruptly declined in rate, leveling off at about 1,000 microorganisms per ml. Differential centrifugation studies showed that most of the staphylococci were promptly situated within leukocytes. In contrast, pneumococci were removed from the blood stream at somewhat slower rates, but had completely disappeared from the blood 90 minutes after injection. During clearance, all of the culturable pneumococci remained free in the plasma layer.

Alterations in circulating granulocytes occurring during blood stream clearance of microorganisms. Total and differential leukocyte counts obtained during clearance studies revealed a striking and selective fall in the number of circulating polymorphonuclear leukocytes after the injection of either staphylococci or pneumococci. Alterations in the level of circulating granulocytes in 8 rabbits receiving either staphylococci or pneumococci are summarized in FIGURE 7.

The injection of either microorganism produced a rapid and profound fall

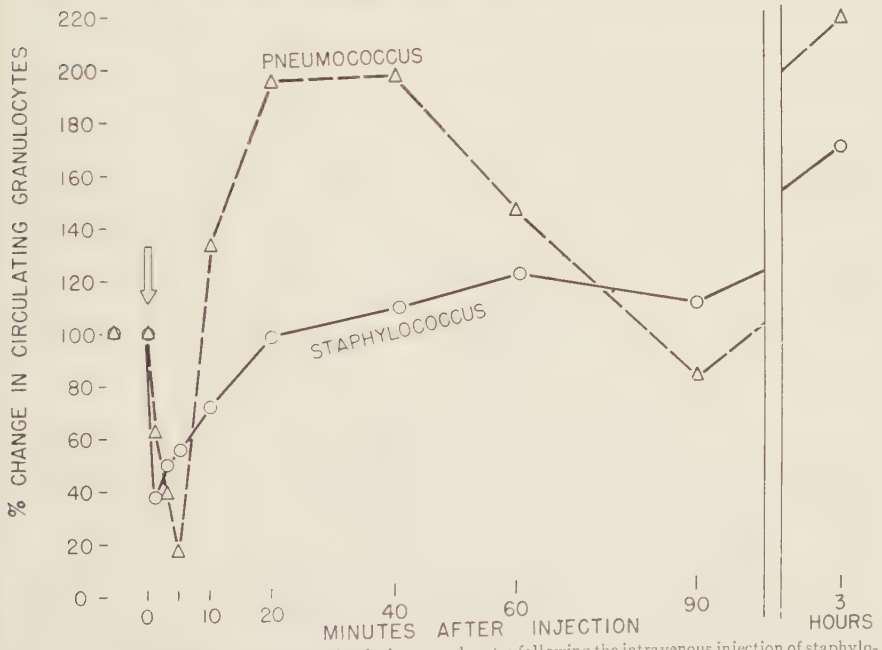

FIGURE 7. Changes in the number of circulating granulocytes following the intravenous injection of staphylococci or pneumococci. Data from 8 rabbits are summarized.

in the number of circulating polymorphonuclear leukocytes. One minute after the injection of staphylococci the number of granulocytes had fallen to 38 per cent of control granulocyte counts. A similar rapid fall occurred following the injection of pneumococci. Leukocytes then swiftly reappeared in the circulating blood, the number of granulocytes equaling or exceeding control leukocyte counts within 10 to 20 minutes following injection. Generally, a leukocytosis subsequently occurred, and increasing numbers of immature granulocyte forms were apparent in smears obtained beyond 20 minutes following injection.

The appearance of intracellular staphylococci on stained blood smears. Careful examination of stained smears revealed that staphylococci could be found within the cytoplasm of granulocytes within 1 minute after the injection of culture. All staphylococci visualized on 10-minute and subsequent smears were contained in cells, and extracellular staphylococci were not found beyond 10 minutes on repeated examinations. Only the pseudoeosinophilic, polymorphonuclear leukocytes of the rabbit appeared to ingest microorganisms.

Similar examination of smears prepared following the intravenous administration of pneumococci also revealed the prompt appearance of intracellular cocci. This finding was unexpected, but appeared in every way to resemble the intravascular phagocytosis of staphylococci. Despite such intracellular location of some pneumococci, the differential centrifugation studies already described failed to reveal the presence of culturable pneumococci in association with leukocytes. It thus appeared probable that such intracellular pneumococci were not longer viable, in contrast to staphylococci, which appeared to survive and remain culturable from within the cytoplasm of leukocytes.

Discussion

The experiments reported in this paper indicate that staphylococci may be swiftly incorporated within rabbit polymorphonuclear leukocytes. Such intracellular residence appears to protect some viable staphylococci from removal in the reticuloendothelial system of the liver and spleen. Following the intravenous injection of staphylococci, the majority of microorganisms are swiftly removed from the circulating blood. As increasing numbers of the circulating microbial population reside within leukocytes, however, splanchnic trapping appears to decline, and splanchnic removal virtually ceases when all of the staphylococci are situated within granulocytes. Saturation of splanchnic removal mechanisms has not been demonstrated in these studies.

The observation that splanchnic mechanisms are once again effective in removing staphylococci from the circulation when extracellular microorganisms reappear during the later phase of increasing bacteremia, suggests that extracellular staphylococci are preferentially sequestered in the reticuloendothelial bed.

The decreasing splanchnic removal of microorganisms noted with the passage of time differs from the findings of Martin and his co-workers[3, 4] who have demonstrated constant rates of splanchnic trapping during a continuous infusion of microorganisms. Such an infusion technique continuously introduces extracellular microorganisms into the blood stream, and it would appear that

the effects of intravascular phagocytosis might not be detected under these conditions.

Circulating polymorphonuclear leukocytes rapidly disappear from the circulation following the injection of microorganisms. This finding has been noted following the injection of a wide variety of substances.[1, 2, 12, 13] Studies by Wood[14] have suggested that the intravenous injection of bacteria may cause alterations in leukocytes and capillary endothelium, resulting in leukocytes sticking to capillary endothelium. Wood's studies have further shown that leukocytes attached to the vascular endothelium are actively phagocytic.

Our observations indicate that staphylococci may be ingested by polymorphonuclear leukocytes during the phase of leukopenia, perhaps by leukocytes sequestered in capillary beds throughout the body. The rapid return of granulocyte counts to preinjection levels within 10 to 20 minutes following injection of bacteria suggests that such sequestered leukocytes may reappear in the circulation containing intracellular microorganisms. Splanchnic removal mechanisms significantly decline or virtually cease coincident with the return of granulocytes to the circulation and the incorporation of most of the circulating staphylococci within leukocytes.

These findings suggest that the intracellular residence of a small fraction of the microbial population following the injection of large numbers of staphylococci may explain the persistence of low-grade staphylococcal bacteremia in rabbits following the phase of rapid removal.

Summary

The studies reported here indicate that a single strain of staphylococci is rapidly ingested by rabbit polymorphonuclear leukocytes. A significant number of granulocytes containing living staphylococci appeared to remain within, or to re-enter the circulating blood.

Intracellular residence appears to protect certain staphylococci from removal from the blood, and trapping of staphylococci during passage through the splanchnic bed virtually ceases when all of the viable circulating staphylococci are incorporated within cells.

It appears probable that the persistent low grade bacteremia observed in rabbits receiving large numbers of staphylococci intravenously is initially due to the residence of living staphylococci within circulating polymorphonuclear leukocytes.

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EXPERIMENTAL STAPHYLOCOCCAL INFECTIONS IN THE SKIN OF MAN

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The concept of virulence of a microbe is relative to a given host species. The fact that an organism is capable of setting up lesions in 1 experimental animal cannot be used as a basis for comparing the virulence of microbial strains in relation to another species of host. The traditional approach of using any convenient laboratory animal in virulence studies may yield completely fallacious findings if different metabolic products in other species of animals contribute to varying degrees of virulence in those species. There is evidence that the virulence of coagulase-positive staphylococci does vary when tested on rabbits, mice, or other animals, but there is no information concerning the existence of similar differences in man (Frappier *et al.*, 1955).

The carrier rate of coagulase-positive staphylococci and their distribution in dust, clothing, and elsewhere is very high, but clinical staphylococcal infection in man is comparatively rare. This suggests that either a minority of nasal strains are endowed with special virulence or that the circumstances of the infection determine the occurrence of disease. Since virulence testing on animals could not be expected to provide the correct answer, it was decided to compare a number of nasal strains with staphylococci isolated from human lesions by injecting volunteers intradermally. All the strains tested were coagulase-positive but, as the other biological properties of such material have frequently been described, no further details were regarded as relevant. Virulence testing in man being necessarily restricted in extent, our primary aim was to establish, within the limitations of the approach, whether or not differences in the resulting lesions could be demonstrated between randomly selected nasal strains of *Staphylococcus pyogenes* and other strains obtained from human lesions, and therefore presumed to be virulent. The indicator effect chosen was pus formation. Since staphylococci typically produce purulent lesions, the minimum inoculum surviving and progressing to pus formation can be used as the basis of comparison between the virulence to man of different strains.

Experiments on man with *Staph. pyogenes* have been carried out before (Garrè, 1885; Bumm, 1885; and Bockhart, 1887), but only to show that they cause disease. No information was available concerning the minimal pus-forming dose or concerning differences between strains. Since the number of volunteers available was insufficient to test an adequate number of strains with different sizes of inocula it was decided to determine the minimal pus forming dose for a strain freshly isolated from a severe lesion and then to compare the effects of the same dose of nasal and of some further known pyogenic strains. If the hypothesis that only a minority of nasal strains are virulent is correct, these strains would be expected not to cause pus formation at that dose.

A strain obtained from an abscess of the neck was injected in varying dilu-

TABLE 1

RESULT OF INTRADERMAL INJECTION OF A FRESHLY ISOLATED PYOGENIC STRAIN (P3). READINGS 48 HOURS AFTER INJECTION.

Volunteers	Dose: 7.5×10^6			Heat-killed control		
	Pus	Swelling	Redness	Pus	Swelling	Redness
A	+	+	+++	−	−	+
B	+++	+++	+++	−	−	+
C	++	−	+	−	−	±
D	+++	+++	++++	−	−	++
E	++	+	+	−	−	±

+ Denotes pus discharging, ± pus not discharging.
− Denotes no reaction.

tions intradermally into 2 young volunteers. This strain was chosen for its high α-lysin production *in vitro* and had the following characteristics: coagulase-postive, orange-yellow in color, mannitol-positive, fibrinolysin-positive, lipase-positive, α-δ-hemolytic pattern, and 7 flocculation lines against Wood 46 antitoxin. Doses of 2×10, 2×10^2, and 2×10^3 cocci in 0.1 ml. obtained by serial dilution of an overnight broth culture caused some redness and a little swelling with the higher doses, but no pus formation. All signs disappeared within 48 hours.

The next experiment, using another strain from an abscess, was done on 3 volunteers at the level of 7×10^4 and 7×10^5 viable cocci. Appreciably more redness and swelling followed, and 1 of the 3 volunteers produced a minute amount of pus with the higher dose. The same strain (P3) was next injected in a 10-times higher dose into 5 volunteers together with a heat-killed control. TABLE 1 shows that, at that level (7.5×10^6 cocci), some pus formation occurred in all the volunteers, with a variable amount of swelling and redness. This was regarded as the minimum average pus-forming dose. The heat-killed control caused neither pus nor swelling, indicating that further *in vivo* growth of the cocci is required to produce the indicator effect. The lesions reached their maximum in 48 hours, discharged, and healed within 7 days. They resembled in appearance small superficial boils. No after effects followed within 9 months of subsequent observation.

Since the minimal pus-forming dose was much higher than would have been expected, the enhancing effect of certain factors was investigated. It was considered possible that the intradermal injection might result in the presence of less plasma than in conditions of natural infection. Two volunteers were injected intradermally with their own plasma. The wheals so produced were then injected with one tenth and one hundredth of the minimum pus-forming dose. The full pus-forming dose was injected at another site as a control. None but the control produced pus, showing that the presence of plasma has no enhancing effect. A similar experiment was carried out with the toxic filtrate of a 24 hour broth culture. This toxin contained only trace of α-hemolysin, since it was incubated without CO_2. Nevertheless, when injected intradermally in doses of 0.1 ml. diluted 1:10 and 1:100, it caused widespread swelling and erythema, and some systemic disturbance. The addition of this

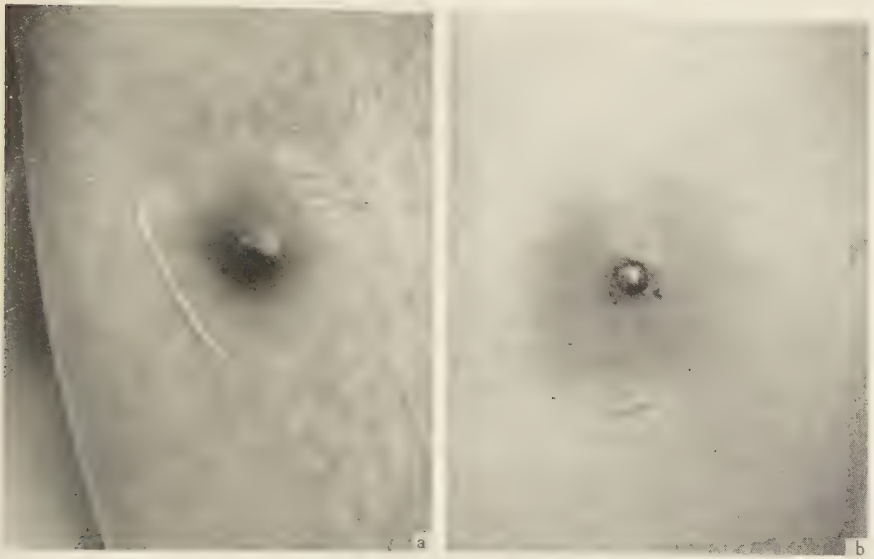

FIGURE 1. (a) Early pustule (24 hours) obtained with 7.5×10^6 staphylococci injected intradermally. (b) such lesions discharged pus after 48 hours. Healing occurred within a week.

toxin to washed staphylococci in a dose of one hundredth of the minimum pus-forming dose failed to cause a pustule in 2 volunteers.

In view of the fact that nasal droplets are believed to play an important part in wound infection, the enhancing effect of mucin was tested. Nasal mucin is known to contain a virulence-enhancing factor (Smith, 1951; and Nungester *et al.*, 1951). The effect of hog gastric mucin of established virulence-enhancing effect in mice was tested on 2 volunteers, using the minimum pus-forming dose as control and one twentieth of this dose suspended in the mucin. No virulence enhancement could be demonstrated in this way, only the control causing pus formation.

In 2 groups of experiments, 5 pyogenic strains were compared with 13 nasal strains. TABLE 2 shows the results with digest broth cultures. Readings were taken after 48 hours and, in this set, only 1 nasal strain failed to produce pus. The dose, accidentally, was slightly lower, but it is noteworthy that this strain appeared to be deficient in several respects. It was coagulase- and lipase-positive but white in color, mannitol-negative, and fibrinolysin-negative, producing Δ-hemolysin only, and no flocculation lines. Some of the strains were tested on 5 volunteers, and 1 strain on 7. The similarity in the reactivity of the volunteers is an interesting finding. In the second set (TABLE 3), washed staphylococci were injected to remove traces of preformed toxin. In this group, 2 anomalous reactions were found. Volunteer O gave no pus formation with any of the 4 strains, although all strains produced pus in the other volunteer. On subsequent inquiry it was found that during the experiment volunteer O had been on Aureomycin treatment for a chest condition. This accidental finding further illustrates the fact that multiplication *in vivo* is necessary

TABLE 2

COMPARISON OF KNOWN PYOGENIC AND NASAL STAPHYLOCOCCI. DILUTIONS MADE FROM 18-HOUR CULTURE IN DIGEST BROTH. READING AFTER 48 HOURS.

Volunteer	Pyogenic No. 3 7.5×10^{7}	Pyogenic No. 4 7×10^{7}	Nasal No. 1 8.5×10^{6}	Nasal No. 2 8×10^{6}	Nasal No. 3 7.5×10^{6}	Nasal No. 4 1.5×10^{6}
A	+		+	+		
B	+		+	+		
C*	+		−	+		
D	+		±	±		
E	+		+	+		
F	+†	+			+	−
G	+†	+			+	−

\+ Denotes pus discharging, ± pus not discharging.
− Denotes no pus.
* Penicillin after 24 hours.
† 6×10^{6}.

TABLE 3

COMPARISON OF SUSPENSIONS OF PYOGENIC AND NASAL STAPHYLOCOCCI, WASHED FREE FROM PREFORMED TOXIN. READINGS AFTER 48 HOURS.

Vol.	P 3 4×10^{6}	P5 2×10^{6}	P6 5×10^{5}	P7 3×10^{6}	N5 3.5×10^{6}	N8 3×10^{6}	N11 7.5×10^{5}	N6 3×10^{6}	N9 3×10^{6}	N12 2×10^{6}	N7 2.5×10^{6}	N10 3×10^{6}	N13 2×10^{6}
L	+												
M	+												
N		+			±			+			+		
O*		−			−			−			−		
P			−			−			−			±	
Q			+			+			±			+	
R				+			±			+			+
S				+			−			+			+

* Volunteer on Aureomycin treatment.
\+ Denotes pus discharging, ± pus not discharging.
− Denotes no pus.

to yield pus. Volunteer P also showed a somewhat similar reactivity, although in this instance we have been unable to elicit a history of antibiotic treatment, and he may represent an individual with a higher natural resistance. The same strains in identical doses caused pus formation in volunteer Q.

It is difficult to visualize conditions of natural infection in which the infective dose is of the order of a million cocci or more. Nasal droplets of a 100 μ diameter, even if they consisted entirely of staphylococci, could contain only 509,000 cocci. This suggests that in the evolution of wound infection the circumstances of the infection rather than differences in virulence of the staphylococci must determine the issue. Like other predatory creatures, staphylococci hunt in packs and, in addition to a large inoculum, there is another way of reaching the critical population required for disease. Delay in the defense mechanism occurs as a result of ischemia induced by adrenaline (Evans, *et al.*, 1948), and a similar mechanism probably renders severely contused tissues prone to infection.

In surgery, stitch abscesses are the commonest forms of sepsis. For this reason we tested the effect of the foreign-body reaction occasioned by a suture. Silk sutures were infected by immersion in serial dilutions of a culture of *Staph. aureus*. They were subsequently dried and the numbers of organisms on them estimated. Sutures carrying 3×10^4 cocci over their entire length were inserted through skin and subcutaneous tissue of 2 volunteers. One stitch was pulled through and removed immediately after insertion, while others were tied and left *in situ*. Subsequent estimation showed that pulling through removed four fifths of the inoculum, leaving this number in the tissues. Nevertheless, no lesions developed at this site. The sutures left *in situ*, however, suppurated, setting up severe local abscesses that required draining and vigorous antibiotic treatment in both volunteers. It was estimated that in this experiment the enhancing effect of the foreign body reaction was at least five hundredfold. In another experiment a single volunteer showed some suppuration around a stitch containing an estimated 100 cocci, corresponding to a potentiation of ten thousandfold. Sterile sutures, used as controls in these experiments simultaneously on the same volunteers, showed no infection. The subcutaneous injection of doses of 10^5 and 10^6 cocci also failed to lead to pus formation.

To sum up, man appears to possess a high degree of natural resistance to *Staph. pyogenes*. Infections of the size to which we are exposed by ordinary hazards are readily eliminated. No differences in the virulence of known pyogenic strains and nasal strains from unselected carriers could be demonstrated by accepting pus formation in man as the criterion of virulence. In surgical infection the foreign body reaction and possibly other mechanisms of delayed defense appear to determine whether clinical disease occurs. In all probability the concept of the critical pus-forming dose also applies to growth in hair follicles and sweat glands, where other factors may allow subsequent entry into the tissues.

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Discussion of the Paper

E. T. Bynoe (*Bacteriological Laboratories, Laboratory of Hygiene, Department of National Health and Welfare, Ottawa, Ont., Canada*): I should like to comment

on the suggestion in Stephen Elek's paper that there is no, or little, difference in the virulence of different strains of coagulase-positive staphylococci, whether from pyogenic lesions or from nasal carriers. For many years, the person with an active staphylococcal infection has been recognized as a more dangerous carrier than the healthy nose carrier. Our D. V. A. special committee has strongly recommended that all infected members of the staff be removed from active duty in the hospital until cured of their infection. In this connection a recent report by Barber and Burston[1] is particularly pertinent. These workers reported that in a maternity unit of a London hospital, of 31 nurses employed in the unit, 55 per cent were carriers of *Staph. aureus*, and that 26 per cent were carrying type 52A. Nearly all the babies (92 per cent) picked up *Staph. aureus* in the hospital, and 45 per cent were carrying type 52A. There were, however, very few infections in the babies during the period of study and these were trivial. Of the 179 babies born during the study period, only 2 had infections due to type 52A, and these infections were minor. A few weeks after the close of the study, however, a nurse came on duty with a boil on her face and, within a few days, there were 2 severe infections in the nursery. Cultures from the nurse with the boil and from the two infected babies were all the same type 52A. Surely there was some difference in the virulence of the 52A strain from the nurse with the boil and the 52A strains from the noses of the healthy carriers!

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THE EFFECT OF ANTIMICROBIAL DRUGS ON AN EXPERIMENTAL STAPHYLOCOCCAL INFECTION IN MICE*

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An essential element in the pathogenicity of any microorganism is its ability to multiply within tissues of an infected host. Techniques developed for the enumeration of viable tubercle bacilli[1] have been applied for the enumeration of viable staphylococci.[2] These methods permitted the measurement of total populations of staphylococci in various tissues of the infected host over long periods of time, both in the natural course of the infection and under the influence of experimentally induced variables.

The purpose of this report is to present studies of the action of antimicrobial drugs on populations of staphylococci subsisting in the kidneys, lungs, and spleens of experimentally infected mice.

Materials and Methods

The Giorgio strain of staphylococci used in these experiments was chosen because of its high degree of virulence for Swiss albino mice and its property of producing penicillinase. It was originally isolated from the purulent exudate of an infected craniotomy wound of a patient at the New York Hospital, New York, N. Y. This strain was coagulase-positive and produced a zone of hemolysis on human and rabbit blood agar plates. It fermented mannite, lactose, sucrose, maltose, and galactose, and was resistant *in vitro* to penicillin concentrations of 3.1 Oxford units per ml. as determined by the standard serial-tube dilution method.

The infecting inocula were prepared from cultures grown in beef heart infusion-peptone broth for 18 hours at 37° C. The infections were induced with 0.1 ml. of culture. This infecting dose was diluted in physiologic saline to a final volume of 0.2 ml. and injected into one of the dorsal tail veins. The number of staphylococci present in the infecting inoculum was determined by inoculating aliquots of appropriate dilutions of the culture in nutrient agar at the time of infection. Calculations were made from plates giving 10 to 100 colonies.

The animals used in these experiments were male and of the CFW strain obtained from the Carworth Farms, New City, N. Y. The weight of the animals ranged from 15 to 20 gm. when they were received at the laboratory, and infection customarily was carried out 1 week after arrival. They were housed in metal cages not exceeding 10 animals per cage and were fed whole or pulverized Wayne Lab-Blox pellets with water ad libitum.

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Drug Administration and Dosage

Aqueous procaine penicillin G (Squibb) was administered daily at a dosage of 1 mg. by the intramuscular route. Streptomycin sulfate (Pfizer) was given by the intramuscular route at a dosage of 4 mg. daily. Novobiocin (Upjohn) was administered in the diet by the thorough mixing of finely-ground food pellets with the amorphous drug in a McLellan dry batch mixer. The dosage of novobiocin was 0.1 per cent of the daily diet.

The microbial enumeration techniques used for the experiments described in this paper are substantially the same as those described by LeMaistre and Sellers[3] and Smith.[4]

Preparation of Tissue Homogenates

Mice were sacrificed with chloroform, and aseptic techniques were utilized in removing organs. Tissue emulsions were prepared by the use of a Teflon homogenizer. All tissues were ground in the presence of distilled water with the exception of those from animals treated with penicillin, in which instance 10 units of penicillinase per ml. of saline was used. The homogenates were found to consist of ruptured tissue cells and intact nuclei.

Organ measurement was achieved by determining the amount of displacement of a measured volume (5 ml.) of diluent (distilled water or penicillinase solution). This figure was ultimately used in expressing the population of staphylococci in terms of unitage (per ml.) of tissue.

Enumeration of Viable Staphylococci

The numbers of living staphylococci present in the infecting inoculum and the homogenates of tissues of infected animals were determined by inoculating nutrient agar with appropriate dilutions of the substance, using the pour-plate method.[3]

Colony counts were routinely performed at 24 and 72 hours after incubation. Two observers made independent plate counts. Utilizing the dilution factors and the number of colonies (preferably in the range of 10 to 100) counted at the appropriate dilution, calculations of the total number of colonies (expressed as viable units of staphylococci) per ml. of tissue were performed. The logarithms of these numbers were plotted graphically as a function of time after initiation of infection. The resulting curves were taken to represent the fate of the staphylococci during the natural course of infection as well as their fate under the influence of treatment with various antistaphylococcal agents.

Considering the dilution that was necessary for preparing the tissue emulsion, there was accordingly a lower limit of this method's ability to detect culturable organisms. The lower limitations varied with the organ size. This method did not permit the detection of fewer than 5 to 10 culturable units of staphylococci per organ.

In experiments concerned with antistaphylococcal drugs, treatment was begun at 2 different times after infection. In certain groups, treatment was started within an hour after infection. In other groups treatment was delayed until 54 hours after infection. The data reflect our total experience up to the

time of this writing, and each symbol depicting treatment groups represents the mean of the tissue populations of 4 to 18 animals. Each symbol from the untreated groups represents the mean of 16 to 36 animals.

Results

Untreated animals. Fifty per cent of untreated animals were dead 7 days after infection, and there were no survivors after 20 days.

The most striking lesions of untreated animals were seen in the kidneys. Gross abscesses were present as early as 2 days after infection. These lesions increased in size and number, maturing into large abscesses that ultimately lead to the destruction of the renal tissue and death of the animals.

In FIGURE 1 may be seen the gross appearance of the kidney lesions from an animal sacrificed 15 days after infection. The diseased kidneys clearly exhibit the swelling and abscess formation seen after 15 days of infection. On microscopic examination the abscesses were seen to be predominantly in the cortex.

This infection did not produce gross lesions in the lungs and spleens. Peribronchial and perivascular cuffing of inflammatory cells were present in the pulmonary tissue. Lymphocytic hyperplasia was seen in the spleens that showed an increase in size.

In FIGURE 2 may be seen the typical behavior of the staphylococcal populations in the kidneys, lungs, and spleens of untreated mice. Each symbol represents the mean of populations from different experiments, and the trend

FIGURE 1. Photograph of mouse kidneys. On the left are kidneys removed from a normal mouse. On the right are kidneys removed 15 days after an intravenous injection of 0.1 cc. of a concentrated culture of penicillin resistant staphylococci (Giorgio). No treatment had been given. Abscesses contained creamy-yellow purulent material.

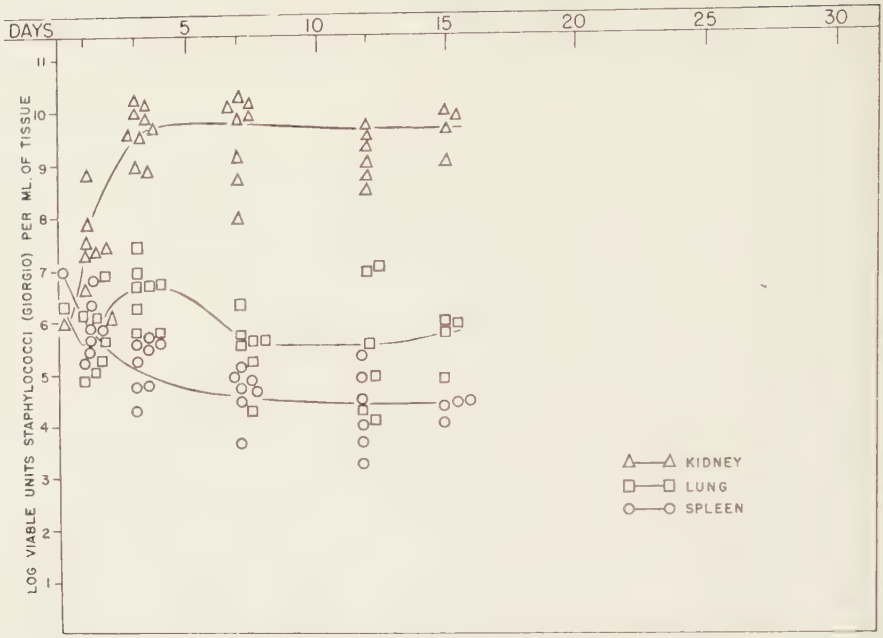

FIGURE 2. Staphylococcal populations (Giorgio) in kidneys, lungs, and spleens of untreated mice. Each symbol represents the mean of 1 experimental group. The initial observations were made 5 minutes after infection. All animals were dead in less than 20 days, and half we re dead in 7 days.

lines thus represent the total experience observed from the untreated control animals in the experiments presented in this paper.

Characteristically, 5 minutes after infection the populations in the kidneys were lower than in the spleens and lungs. A marked rise was then seen in the staphylococcal census of the kidneys. After the third day of the infection there occurred a stabilization of the populations. The lung populations showed a rise on the third day of infection after an initial fall. A stabilization of the staphylococcal census was seen during the second week of the infection. A sharp initial fall in the splenic populations was seen during the first week of infection, which was followed by a stabilization.

The differences seen in the viable populations of staphylococci in these 3 organs were of a qualitative nature only during the first week of the infection. During the second week there was a stabilization of the microbial census, but at different population levels.

Penicillin and streptomycin. In FIGURES 3, 4, and 5 may be seen the effects of penicillin and streptomycin administered singly and concurrently.

The stock strain Giorgio was susceptible *in vitro* to streptomycin concentrations of 0.8 μg. per ml. As stated above, it was resistant to penicillin concentrations of 3.1 units per ml.

As seen in FIGURE 3, the populations in the kidneys of control animals followed the pattern described above, with mean census levels of over 1 billion organisms per ml. of kidney attained 3 days after infection. Penicillin alone

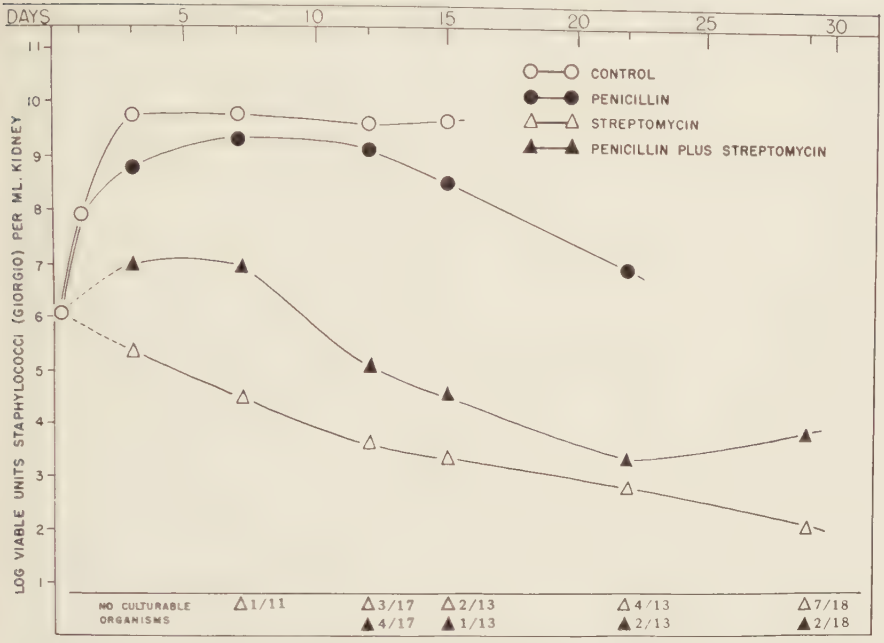

FIGURE 3. Influence of penicillin and streptomycin used singly and together on penicillin-resistant staphylococcal populations (Giorgio) in mouse kidneys during 28 days of therapy. Each symbol represents the mean of the bacterial populations of animals from several experiments (see text). The techniques used permitted detection of no less than 5 to 10 staphylococci per organ.

demonstrated activity of a low order in causing a slight reduction in the bacterial census and increasing the survival time of the animals. Whether the fall in populations after 2 weeks of treatment was a function of penicillin action, a mobilization of host defense mechanisms, or a combination of both is a matter for speculation.

After the legend "No culturable organisms" is seen the incidence of animals whose tissue populations have been reduced below detectable levels. In animals given streptomycin alone, a fall in total bacterial population in the kidneys began at once and continued throughout the treatment. The populations were reduced below detectable levels in some of the animals as early as the first week of treatment.

It was of particular interest, however, that staphylococci were seen to persist in a majority of the kidneys after 28 days of treatment. Most of these persisting microorganisms were susceptible upon *in vitro* subculturing to streptomycin concentrations of less than 1 μg. per ml. The kidney populations from animals treated with streptomycin plus penicillin are depicted by the filled-in triangles. The concomitant use of penicillin and streptomycin was significantly less effective in reducing the staphylococcal populations than was streptomycin when used alone. This was evidenced not only by the mean bacterial census but also by the incidence of animals in which no organisms could be cultured.

In the lung (FIGURE 4) the control curve showed the initial fall, subsequent

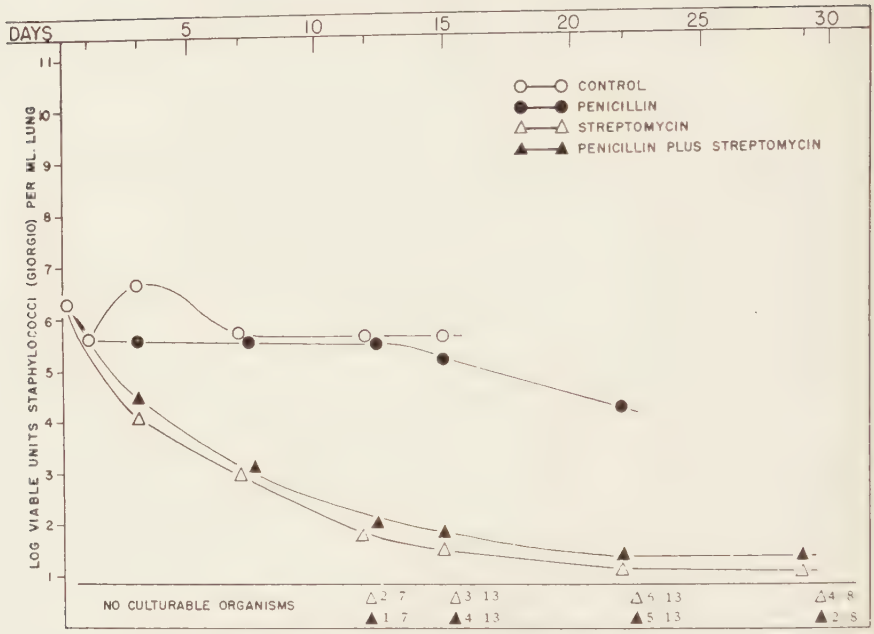

FIGURE 4. Influence of penicillin and streptomycin used singly and together on penicillin-resistant staphylococcal populations (Giorgio) in mouse lungs during 28 days of therapy.

rise, decline, and stabilization of the bacterial census previously described. Penicillin alone was effective in preventing the initial rise seen in the untreated animals. Streptomycin was effective in reducing the lung populations to very low levels after 2 weeks of treatment. This was also reflected in the incidence of animals from which no staphylococci could be cultured. At each observation time the lung populations of animals treated with streptomycin plus penicillin were slightly higher than those from animals treated with streptomycin alone. This may be reflective of the same antagonism that was seen in the kidneys of the same animals. Six of 8 animals in the combined-treatment group and 4 of 8 animals in the streptomycin-alone group had persisting staphylococci in the lungs after 4 weeks of treatment.

The splenic populations from the same animals are shown in FIGURE 5. As described previously, the control curve was not seen to rise even during the first week of infection, as in the kidneys and lungs. Instead there was a sharp fall during the first week, followed by a stabilization in the bacterial census. Penicillin again was seen to have a definite but low order of effectiveness. Both streptomycin alone and streptomycin plus penicillin effected a marked reduction in the splenic populations after 1 week of treatment. The opposite effect of that observed in the kidneys and lungs of the same animals was seen in the splenic population of animals treated with streptomycin plus penicillin. The bacterial census from animals treated with both drugs was always slightly lower than that seen in those treated with streptomycin alone. There were persisting staphylococci in animals treated with streptomycin alone and in those

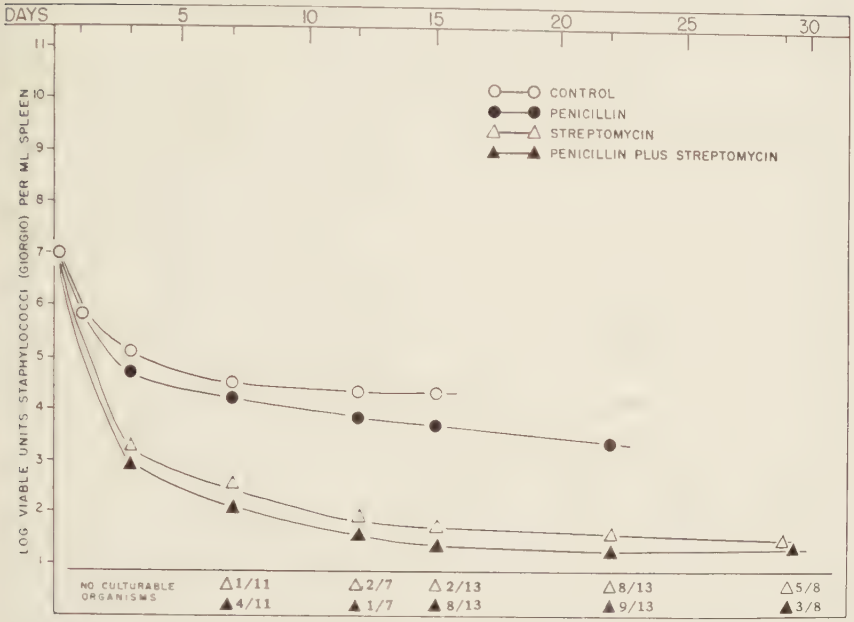

FIGURE 5. Influence of penicillin and streptomycin used singly and together on penicillin-resistant staphylococcal populations (Giorgio) in spleens of the same animals whose lung populations are shown in FIGURE 4.

treated with streptomycin plus penicillin after 28 days of treatment despite the very low bacterial census attained with these regimens.

Penicillin and streptomycin—54-hour infection: In FIGURE 6 may be seen the effects of penicillin and streptomycin used singly on staphylococcal populations in the kidneys when treatment was delayed until 54 hours after infection. At this time macroscopic abscesses were seen in the kidneys. No abscesses were seen in the lungs and spleens of the same animals.

The control curve showed the marked rise in the staphylococcal populations described above. At the time treatment was started the microbial census had exceeded a billion organisms and grossly visible abscesses were present. Penicillin exhibited moderate activity in reducing the bacterial census and prolonging the survival time of the animals a week longer than the untreated animals. Of particular interest were the observations made in the streptomycin-treated animals. A marked drop occurred during the first 10 days of treatment. After this initial fall the bacterial census stabilized and persisted at a level of approximately 1 million organisms throughout the remainder of the experiment. This was contrasted with the effect streptomycin was seen to exert in reducing the microbial populations to low levels when treatment was started within an hour after infection.

Despite the initial fall seen in the staphylococcal populations of streptomycin-treated mice in the 54-hour infection, the lesions increased in size. By the 19th day of treatment the lesions had decreased in size, and fibrotic changes

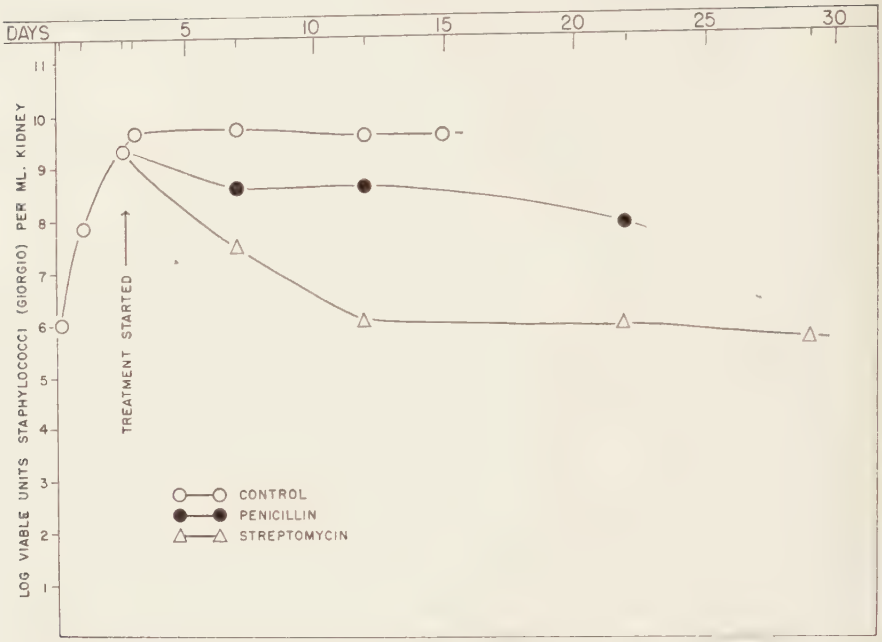

FIGURE 6. Influence of penicillin and streptomycin on penicillin-resistant staphylococcal population (Giorgio) in mouse kidneys when treatment was started 54 hours after infection. At the start of therapy, macroscopic lesions were visible in the cortices of all kidneys examined.

were seen to take place. On the last observation day, after 26 days of treatment, considerable scar formation was present.

Novobiocin, penicillin, streptomycin. In FIGURE 7 are seen the effects of novobiocin and penicillin used singly and together and novobiocin plus streptomycin on kidney populations when treatment was started within an hour after infection. The parent Giorgio strain of staphylococci was susceptible *in vitro* to novobiocin concentrations of 0.2 μg. per ml.

The populations from the control and penicillin-treated animals were similar to those described above. An initial increase in the staphylococcal populations was seen after 3 days of treatment with novobiocin alone. At this time, abscesses were grossly evident in the kidneys. These lesions gradually disappeared and were replaced by scar tissue during the latter 2 weeks of the experiment.

It should be noted here that the same phenomenon was observed in kidneys of animals treated with erythromycin and oxytetracycline.[7] Namely, when the drug and infection were started together, the staphylococcal populations in the kidney increased and lesions were seen to develop. After the initial population increase a reduction in the microbial census ensued and continued to fall during the 4-week observation period.

Novobiocin plus penicillin was more effective than streptomycin alone in reducing the populations during the first 12 days of treatment, whereas a sig-

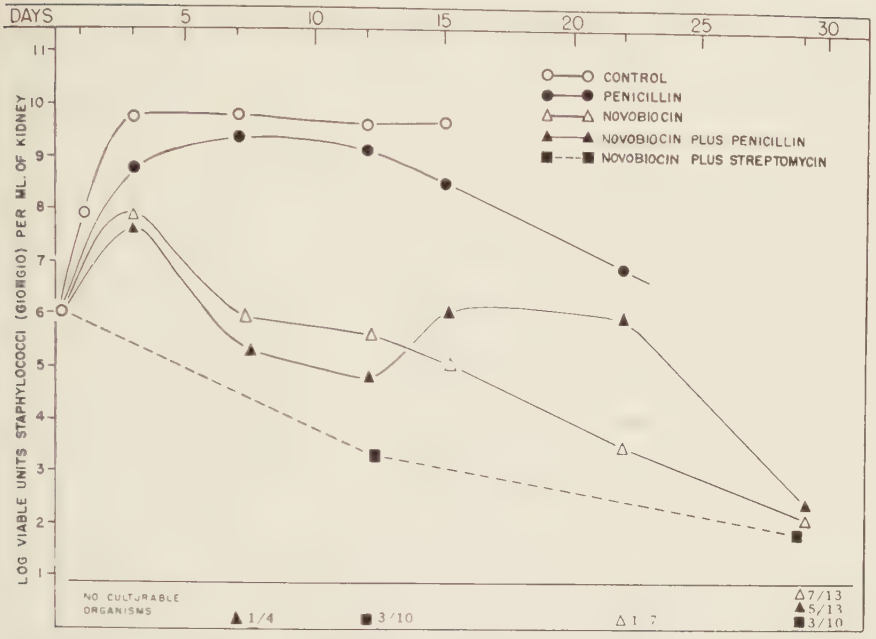

FIGURE 7. Influence of drugs on penicillin-resistant staphylococcal populations (Giorgio) in mouse kidney after 28 days of therapy. The comparative effects of penicillin and novobiocin used singly and together and of novobiocin plus streptomycin are shown.

The trend line of novobiocin plus streptomycin is broken since observations were made only at 2 points in time.

nificant degree of antagonism was seen on treatment days 15 and 22. This antagonistic action was seen in the lungs and spleens of the same animals and was similar to that seen in animals treated with streptomycin plus penicillin, described above. After the third week the kidney populations from animals receiving novobiocin plus penicillin fell, and staphylococci could not be cultured from 5 of 13 animals tested on the last day of the experiment.

After 2 weeks of treatment the microbial census from the kidneys of animals treated with novobiocin plus streptomycin was lower than the other treatment groups.

Again it was of particular interest that, despite the low populations in the various treatment groups after 4 weeks of therapy, staphylococci persisted in the kidneys of 45 to 70 per cent of all remaining animals tested at this time.

Discussion

Microbial enumeration techniques were used to measure penicillin-resistant staphylococcal populations in various tissues of mice, in the natural course of the infection, and under the influence of antistaphylococcal drugs. These studies have yielded a number of features worthy of comment.

This experimental system produced a situation in which, in the same un-

treated animals, a marked degree of multiplication of populations was seen in the kidneys, only a slight population increase was seen in the lungs, and a declining population was seen in the spleens. The high population levels in the kidneys were associated with the formation of abscesses that led to the destruction of the renal tissue and death of the animal. No abscess formation or necrosis of tissue were seen in the lungs or spleens.

It was of interest that after the first week of infection there was a stabilization of the microbial census in all 3 tissues but at different population levels. Previous studies with tuberculous animals, in which the same techniques were employed, demonstrated a somewhat similar situation in the lungs and spleens.[6] A stabilization of the populations of tubercle bacilli occurred in the spleens 1 month after infection and, in the lungs, after 2 months. Another similarity of these 2 infections was seen in the kidneys of animals infected with staphylococci and in the lungs of those infected with tubercle bacilli. In each situation lesions would continue to progress and destroy the organ for a substantial period after the microbial census had stabilized. It was believed that in each of these situations the lesions progressed as a function of rapidly multiplying organisms. It is suggested that the reason this was not reflected in the population curves of viable bacteria is that bacterial death occurred at a rate nearly that of bacterial multiplication.

It was of considerable interest that penicillin therapy in this penicillin-resistant staphylococcal infection exerted effective action in lowering the microbial census in all 3 organs studied, and that it was a factor in prolonging the survival time of the animals. The infecting Giorgio strain was resistant *in vitro* to penicillin concentrations of 3.1 Oxford units per ml. as determined by the standard serial-tube dilution method. The dosages of penicillin used in these experiments produce blood concentrations well below this range. A reasonable explanation for this apparent paradox is that the infecting inoculum contained an admixture of highly susceptible cells as well as resistant ones. *In vitro* experiments demonstrated this to be so.

When therapy was started at the time of infection, certain differences were observed in the streptomycin- and novobiocin-treated animals. A population increase occurred during the initial 3 days of novobiocin treatment, whereas an immediate reduction of the microbial census was seen in the tissues of the streptomycin-treated animals. Kidney lesions were seen to develop during the first week of treatment with novobiocin, but were not seen in the streptomycin-treated animals. Throughout the first 3 weeks of treatment streptomycin effected a greater reduction of the staphylococcal population than that seen in the novobiocin-treated animals. After 4 weeks of treatment, however, the population levels seen in the tissues of each group were comparably low.

When penicillin was administered concomitantly with streptomycin or novobiocin in this penicillin resistant infection, antagonism was consistently seen in some phase of the experiments. This phenomenon was seen throughout the 28-day treatment period in the streptomycin-penicillin animals, but only in the latter phase of treatment in the novobiocin-penicillin animals. Antagonism was most striking in the kidneys of the streptomycin penicillin animals, whereas

it was not in evidence in any phase of treatment in the spleens of the same animals. Thus it was seen that antagonistic action and additive effects were observed in the same treatment groups at different times, and 1 effect could be present in 1 organ while just the opposite effect could be seen to take place in another organ of the same animal at the same time.

When the infection was allowed to progress until kidney lesions were established before starting therapy, the effectiveness of streptomycin action was significantly reduced in lowering the renal staphylococcal populations. This was in contrast to the highly effective action seen in the lungs and spleens of the same animals where no abscess formation was present and to the action seen in all 3 organs when treatment was started at the time of infection.

Of major interest was the fact that, despite the degree of effectiveness exhibited by any of the drugs used either singly or in combination, persisting staphylococci were present in a majority of the animals after 28 days of therapy in the kidneys, spleens, and lungs. The emergence of drug resistance in the usual sense did not explain the presence of these persistent staphylococci.

Summary

Microbial enumeration techniques were used to study the fate of penicillin-resistant staphylococci in the kidneys, lungs, and spleens both in the natural course of the infection and under the influence of antistaphylococcal regimens.

This experimental system produced a situation in which, in the same animals, a marked degree of multiplication was seen in the kidney, only a slight population increase was seen in the lungs, and a declining population was seen in the spleens of untreated animals. The high population levels in the kidneys were associated with the formation of abscesses.

A consistent observation was the finding of persisting organisms in all treated groups after 28 days of therapy. The emergence of drug resistance did not explain the presence of these persistent staphylococci, as a large proportion of them were found to be susceptible when tested *in vitro* against the drugs used for therapy.

When the infection was allowed to progress for 54 hours, until kidney abscesses were established, the effectiveness of streptomycin in lowering the kidney populations was significantly reduced.

Penicillin alone exhibited definite effects in lowering the bacterial populations and increasing the survival time of mice infected with this penicillin-resistant staphylococcal strain.

Antagonism was consistently seen in some phase of all experiments when penicillin was used concomitantly with the other drugs.

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Part II. Biological Characteristics of Staphylococci that May Relate to Virulence

BIOLOGICAL CHARACTERISTICS OF STAPHYLOCOCCI RECOVERED FROM PATHOLOGIC MATERIALS

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When considering the biological characteristics of staphylococci from human pathologic material, it is important to bear in mind that strains recovered within a hospital may represent a selected group that happen to be endemic in the wards. In the orthopedic hospital housing my laboratory, 61 strains of staphylococci obtained from new patients who had been infected before admission belonged to 18 different phage types whereas, out of 25 strains from infections within the hospital, 22 belonged to the same phage type.

My associates and I have studied 1300 strains of staphylococci from sources such as osteomyelitis, secondarily infected tuberculous sinuses, and urinary infections. I shall describe briefly our methods and our findings.

Coagulase. All were shown to produce coagulase *in vitro* when tested with human plasma. We found that the plate method, in which 12 per cent v.v. human plasma in nutrient agar is poured into Petri dishes at 45° C., was a satisfactory alternative to the tube test. Zones of opacity develop around spot inoculations overnight at 37° C.

Fibrinolysin. This plate method also allows the demonstration of fibrinolytic strains after further incubation, as the opaque rings formed by coagulase are cleared by fibrinolytic strains. Demonstration of fibrinolysis is more clear-cut when the same plasma-agar mixture is held at 56° C. for 20 minutes before pouring onto plates. This gives an opaque medium on which fibrinolytic colonies produce clearing overnight. FIGURE 1 illustrates, on the left, the clearing of coagulase opacity by fibrinolytic strains on the plasma-agar plate poured at 45° C., designated the fibrinogen plate; and, on the right, the clearing by the same strains of the opaque plasma-agar plate, designated the fibrin plate, which had been heated to 56° C. Further separation of fibrinolytic strains into those that produce staphylokinase and those that produce staphylococcal protease may be achieved by adding soybean trypsin inhibitor to the medium. This inhibits the staphylokinase-activated plasmin or fibrinolysin, but does not inhibit the staphylococcal protease.

I have described these plate methods in some detail because we have found them useful in survey work. Our plasma was obtained from outdated blood-bank blood, and was usually satisfactory, though some batches were deficient in cofactor or plasminogen. Reconstituted dried plasma was useless. In our series, about 70 per cent of the strains were fibrinolytic and, in the majority of these, fibrinolysis was inhibited by trypsin inhibitor — that is, their proteolytic activity under the conditions of the test was due to activation of plasmin, not to their own protease.

FIGURE 1. On the left is a plasma-agar plate poured at 45° C. with colonies of *Staph. aureus* that have incubated for 48 hours. After overnight incubation these colonies had opaque rings around them due to the diffusion of coagulase. Further incubation has led to the clearing of these opaque zones around strains 1, 4, 7, 8, 9, and 10.

On the right is a plasma-agar plate on which the fibrinogen has been rendered opaque by heating at 56° C. for 30 minutes before incorporation into the agar medium. Zones of clearing around the colonies after overnight incubation correspond to the clearing of the coagulase zones on the other plate.

Reading: All strains produce coagulase, but strains 2, 3, 5, and 6 are not fibrinolytic.

Clumping factor. The slide method of Cadness-Graves *et al.*[1] was considered a useful screening method for detecting coagulase-producing strains, but recent work by Duthie[2] gives a different interpretation to this test. Duthie has shown that most pathogenic strains of staphylococci will clump in fibrinogen solution, and that this clumping does not require the presence of the cofactor required for coagulase clotting. He suggests that staphylococci may absorb fibrinogen, and that the free negatively charged groups of the absorbed fibrinogen may then unite with the positively charged groups on other cells, causing clumping. Staphylococcal antiserum added to coagulase-positive staphylococci will prevent both the absorption of fibrinogen and the clumping. It so happens that most strains that produce free coagulase also produce the clumping factor, but they appear to be distinct agents, which may explain why Williams and Harper[3] found that 12 per cent of strains, coagulase-positive by the tube test, were negative by the slide test. As our strains were primarily selected by the slide test, they were all positive.

Hemolysins. As there is not space to discuss the hemolysins individually, I shall restrict myself to some observations about the α-lysin which has been regarded as the most important in human infection. Some workers have found a complete correlation between α-lysin production and pathogenicity, and it seems pertinent to discuss this finding here in the light of the preceding paper by Rogers. For example, Marks,[4] in a study of staphylococci from skin infections in coal miners, found that only 4 per cent of coagulase-positive strains did not produce α-lysin, and that every one of 100 strains definitely responsible for human infection produced α-lysin. Marks concludes that the production of α-lysin is a more accurate and convenient criterion of pathogenicity of staphylococci than the production of coagulase. In contrast to this, in our series[5] approximately 18 per cent of strains from human sources did not produce α-lysin on primary isolation, though some did so subsequently. Table 1 shows the distribution of the various hemolysins in 49 strains from osteomyelitic lesions. In this series 18.5 per cent do not produce α-lysin. Table 2 shows the range of anti-α-hemolysin titers in 259 patients, and we see that more than half of the patients with staphylococcal osteomyelitis show no rise in this antibody.

Table 1

Distribution of Properties of Strains of Staphylococci from Cases of Osteomyelitis

Spectrum	No. of strains	Percentage
CFADH	16	32.6
CFAH	9	18.4
CAH	6	12.2
CADH	6	12.2
CFH	4	8.2
CH	4	8.2
CFABDH	3	6.1
CFBH	1	2.1
Total	49	100.0

Symbols: C = coagulase; F = fibrinolysin; A = α hemolysin; B = β hemolysin; D = δ hemolysin; H = hyaluronidase.

Table 2
Anti-α-Hemolysin Titers of Human Sera

Anti-α-hemolysin titer in units per ml.	Type of case		
	Osteomyelitis	Secondary staph. infection	No. staph. infection
>2	30	20	140
2–4	9	6	23
4–8	5	7	5
8–16	6	4	1
16–32	1	1	1
	51	38	170

The response to toxoid proves that this absence of antibody response is not due to an incapacity to produce antibody. I am not convinced that the substance that lyses rabbit cells is identical with the necrotizing toxin, but assuming that α-lysin and this toxin, if not identical, are closely associated, it seems to me that there are 2 important factors to consider when assessing the role of such a toxin in infection. First, selection pressure on variants or mutants may vary not only with conditions at the time of infection but may vary as a consequence of infection. Second, the effect of by-products of the pathological process may influence the production and action of toxins.

Mark's patients had acute infections of the skin, and it may be true that α-lysin is a necessary armament for the initiation of infection by staphylococci in the intact skin. In our series, about 25 per cent of the strains were obtained from postoperative infections where foreign bodies such as sutures, steel pins, and foreign grafts have probably had the effect, as Stephen Elek describes elsewhere in these pages, of greatly reducing the efficiency of host defense. In such circumstances toxin production may not be necessary for the initiation of infection.

About half of our strains were from chronic infections of bones and joints and of tuberculous sinuses. In such environments nontoxigenic strains may become predominant as a result of the absence of selection pressure. Coagulase-negative nontoxigenic staphylococci have been recovered from the most chronic type of staphylococcal infection, "Brodie's abscess."

But the overgrowth of nontoxigenic variants is not the only explanation for this difference in toxin production in acute and chronic infections. H. J. Rogers'* suggestion that *in vivo* natural macroanions such as chondroitin sulfate, hyaluronic acid, heparin, and the nucleic acids may inhibit the formation of enzymes, hemolysins, and other active proteins, and also stop their action when formed, provides a second explanation. Our failure on occasion to find α-lysin from staphylococci on primary isolation from bone lesions, and the absence of detectable antibody in such a high proportion of patients with such infections, may be due in part to the inhibition of α-lysin by macroanions, and the accumulation of these may be due to the pathological process. In other words, the toxic action of staphylococci may depend partly upon the presence

* See "The Formation of Extracellular Enzymes by Staphylococci" by H. J. Rogers, p. 132.

or absence of inhibitors such as these macroanions in the immediate environment, and this no doubt varies in the body from one site to another just as it may vary in time during the course of an infection.

Elek and Levy[6] have reported that a high proportion of coagulase-negative strains produce nonspecific hemolysis of rabbit and sheep cells. They suggest that the use of the term "hemolytic staphylococcus," implying pathogenicity, is to be deprecated as meaningless, and with this I agree.

Hyaluronidase. There has been some speculation about the role of hyaluronidase in staphylococcal infection. This enzyme is produced by most if not all pathogenic strains, but it has been pointed out by A. A. Miles that an early dispersal of staphylococci may be disadvantageous to the organism. It is possible that the role of hyaluronidase as a "spreading factor" is a red herring, and that this enzyme, by depolymerizing hyaluronic acid in connective tissue, is removing a potential inhibitor. Other enzymes that might remove natural inhibitors should be sought.

Leukocidins. The identification of the leukocidins with α- and β-hemolysins has been postulated but neither of these has been purified. There is no clear correlation between leukocidin and pathogenicity, and we await their purification for more precise knowledge of their actions.

More work is needed on the purification of the lysins and other antigens and on their action in man. Howard[7] has found that the number of antigen-antibody flocculation lines is directly related to the virulence of different strains for mice, the number varying from 8 in the most virulent to 0 in the avirulent strains. In our own studies, greater pathogenicity was associated with a broad spectrum of toxins rather than with greater production of any one toxin.

Conclusions. To sum up, there are a number of tricks that we can make the *Staphylococcus* perform in the laboratory, such as clotting fibrinogen, lysing rabbit and sheep cells, dissolving fibrin or mucin, and fermenting mannite but the more one works with *Staphylococcus* the less fruitful this systematic approach appears to be.

Personally I doubt whether coagulase is an important factor in pathogenicity, but I suspect that the association of coagulase production with greater resistance to neutrophil lysozyme is important. I have not found any evidence for *in vivo* clotting by coagulase. It has been argued that coagulase clotting is a defense mechanism of the *Staphylococcus*, protecting it from phagocytosis, and of the host, protecting the body from invasion, but teleological arguments of this sort do not help us.

Protease and hyaluronidase do not appear to be important as spreading factors in uncomplicated infections, though when staphylococci are present with other organisms such as vaccinia virus[8] or tubercle bacilli,[9] hyaluronidase may contribute to the synergism of these double infections by its spreading action. Both these enzymes, however, probably alter the milieu in other more complicated ways.

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PROBLEMS IN THE COAGULATION OF PLASMA BY STAPHYLOCOAGULASE*

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The identification of the participating factors is a prerequisite for the elucidation of the mechanism of the clotting of plasma by staphylocoagulase. The characterization of staphylocoagulase had to await the development of methods that favored its elaboration in high concentration in the culture medium. With such cell-free supernatants as the starting point, purification and concentration proved feasible. A second major area of interest has been the nature of the plasma factor that participates in the reaction, hereafter to be designated as the coagulase-reacting factor, or CRF. Does the coagulase-clotting completely bypass the primary steps of the physiological mechanisms that eventuate in the conversion of prothrombin to thrombin? Does coagulase act directly on fibrinogen, thromboplastinogen, prothrombin, or on some wholly distinct plasma component? These questions have been particularly pertinent since, contrary to physiological coagulation, coagulase acts in the presence of citrate and oxalate ions and of heparin.

Concepts of the mechanisms involved in physiological clotting are undergoing rapid change.[1-3] Many new factors have been described, and their functions and relationships to each other are not yet fully established. Lacking a stable base line for comparison, any analysis of the mechanisms of coagulase-clotting must be regarded as tentative at the present time, but certainly such analysis is contingent upon the identification of the involved factors. Since other contributions to this monograph deal with many diverse aspects of coagulase, no attempt will be made to review the total field. The purpose of this presentation is, rather, to summarize work in progress and previously reported data upon which the identification of the factors involved in coagulase-clotting may be based.

Staphylocoagulase

During the many years that coagulase was considered inseparable from living staphylococci, little progress in establishing its nature could be anticipated. After Walston[23] had demonstrated that coagulase may be recovered from cell-free supernatants of appropriate cultures, the way was paved for studies aimed at purification and characterization. Two hundred and forty-two strains of staphylococci were tested quantitatively for cell-free coagulase upon cultivation in brain-heart infusion broth (Difco) to which a mixture of trace ions had been added.[25] While approximately 50 per cent of the strains exhibited coagulase activity only when living cultures were used, about 9 per cent yielded high concentrations of coagulase in the merthiolated cell free supernatants, as deter-

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mined by their ability to clot noninhibitory plasma in 24 hours at coagulase dilutions of 1:1280 to 1:20,480. Using 1 such potent coagulase producer, strain no. 104, extensive purification studies were undertaken.[24] After precipitation of the active principle at a pH of 3.8 to 4.0, purification was carried out by several cycles of alcohol treatment in the cold under controlled pH, alternating with the removal of impurities by the use of low concentrations of ammonium sulfate. A variety of alternative procedures tested in our laboratory have yielded less satisfactory products.

Although the products obtained were purified about three hundredfold, they were not completely homogeneous on electrophoresis and in the ultracentrifuge. One preparation had an uncorrected sedimentation rate of 1.24 for the principal symmetrical component that represented well over 95 per cent of the total material. The purified coagulase was nondialyzable through Visking membranes and yielded nitrogen values of 14 to 16 per cent. Chromatography of hydrolyzed coagulase by the Dent[1] technique, carried out in our laboratory by Margaret Drummond, was consistent with the presence of the following amino acids: aspartic acid, glutamic acid, serine, glycine, threonine or lysine, arginine, hydroxyproline or tyrosine, histidine, and tryptophane or valine. In contrast to the thermostability of crude coagulase, purified preparations may lose over 90 per cent of activity when heated at 65° C. for 30 minutes and are all but totally destroyed by boiling for 15 minutes. Purified coagulase also was labile when diluted in water or in saline, and it was rapidly inactivated by superoxol and by ascorbic acid. Crystalline trypsin, chymotrypsin, and plasmin all inactivate coagulase, while calf-thymus peptidase is inert. All the evidence, therefore, points to the protein nature of staphylocoagulase.

Although coagulase has been generally considered nonantigenic,[10] this view must be abandoned. It is now well established that staphylocoagulase is antigenic for the rabbit,[2, 27] monkey,[16] rarely the chicken[16] and, presumably, for man.

Few detailed observations have been carried out on *in vivo* effects of highly purified coagulase.[24] When this product was administered intracutaneously and subcutaneously to rabbits there was a striking lack of local reactivity, in sharp contrast to the dermonecrosis of the α-lysin. When, however, excessive doses were administered to rabbits intravenously, prompt death of the animals resulted. In some of these animals the blood withdrawn immediately after death remained fluid, and no fibrinogen was demonstrable by the protamine test. While it is tempting to ascribe this action to the *in vivo* action of coagulase, one must note that similar effects may be induced by crude tissue extracts and other preparations.[36]

In titrating for coagulase, several precautions must be observed. When serial dilutions of coagulase are made in the presence of a constant amount of undiluted plasma, it is well to use 2 per cent peptone saline as the diluent to avoid the deterioration of coagulase at higher dilutions. It is also necessary to select noninhibitory human or rabbit plasmas. In a study of 307 unselected human plasmas[28] it was noted that less than 50 per cent were sufficiently noninhibitory to merit use in the coagulase titrations by this technique, while Lominski and Roberts[9] reported that 212 of 348 samples of human sera inhibited

Table 1
The Clotting of Plasma by Coagulase

Clotting time (Min.)	Coagulase concentration (Reciprocal)
5	200
10	500
20	1,120
40	2,240
80	4,480
160	8,960
320	17,920
640	35,840
1,280	61,440

the coagulase-plasma reaction. Using potent purified coagulase, making dilutions in 2 per cent peptone-saline, and adding an equal volume of noninhibitory plasma, the clotting time is inversely proportional to the concentration of the coagulase.

The Coagulase-Reacting Factor of Plasma

It is well to bear in mind that the titration of CRF activity has not been standardized and that different laboratories cling to their preferred methods of assay. In general, the time for a fibrin clot to appear has been taken as the end point. Regardless of the method of titration, certain factors may influence the end point profoundly and must be carefully controlled. As already indicated, the potency of the coagulase preparation is of critical importance. Second, the occurrence of inhibitory zones at the more concentrated levels of plasma, whether due to antibodies or to nonspecific inhibitors, must be taken into account. Third, adequate fibrinogen must be supplied at the higher plasma dilutions. Finally, clotting due to thrombin-fibrinogen interaction must be controlled. In our earlier studies[25, 26] it had proved expedient to titrate CRF by observing the evolution of fibrin up to 24 hours upon reacting several concentrations of coagulase with tenfold dilutions of plasma. Subsequently,[30, 33, 35] titrations have been carried out with a single concentration of coagulase and suitable CRF dilutions to give rise to fibrin clots within 15 to 20 minutes.

Although it is necessary to modify the concentration of reagents for specific studies, a representative scheme of titration may be taken as follows:

To 0.2 ml. aliquots of CRF dilutions in either veronal-citrate-albumin buffer or in saline containing 2 mg. of bovine albumin per ml. were added 0.2 ml. of bovine fibrinogen, and the stop watch started as 0.2 ml. of coagulase was introduced. The appearance of the first wisp of fibrin was taken as the CRF clotting time. The CRF dilutions were frequently doubled, starting with 1:100. The coagulase was generally of such potency as to clot an equal volume of undiluted noninhibitory plasma within 20 seconds.

In 1944, Smith and Hale[39] reported their significant study on the nature of the plasma factor reacting with staphylocoagulase. They concluded that the plasma factor was neither fibrinogen nor prothrombin since, in their hands, plasma

treated by the Mellanby technique of dilution and acetic acid precipitation at a pH of 5.3 failed to react with coagulase. They concluded further that coagulase may be similar to prothrombin and may require "activation" by a plasma factor. These workers felt, however, that the alternative possibility of the plasma factor being the substrate was not wholly ruled out. In the hands of other investigators the findings of Smith and Hale that a plasma-reacting factor existed were confirmed, although varying conclusions were reached concerning its nature and localization.[2, 5, 7, 11, 15, 25, 26]

As the first approach to a more precise identification of CRF, extensive fractionation procedures were undertaken.[25] Contrary to the findings of Smith and Hale, it was found that Mellanby precipitates did react with staphylocoagulase. Ammonium sulfate fractionation and the quantitative titration of CRF indicated maximal concentration between 33 and 50 per cent saturation, with a peak at 45 per cent saturation. There was considerable dispersion of activity, however, and even the albumin fraction was contaminated with CRF. When, therefore, plasma fractions prepared by controlled ethanol precipitation were made available by the Department of Physical Chemistry of the Harvard Medical School, Boston, Mass. an attempt at a more precise localization of CRF activity was sought. In agreement with Smith and Hale and with others,[7, 11] but contrary to the recent report of Frederiq,[3] fibrinogen was eliminated when it was shown that only crude fraction I (the principal fibrinogen fraction) was rich in CRF, while a purified preparation indicated that the CRF was found in the fibrinogen-free component. Similarly, albumin (fraction V) was free of CRF. The globulins, however, principally nonthrombin-clotting fractions I, III-1, III-2, and IV-1 retained considerable CRF activity. The tests of plasma fractions prepared under controlled alcohol precipitation, therefore, successfully eliminated fibrinogen and albumin from further consideration but did not exclude prothrombin and, possibly, other blood-clotting factors as contenders for identification with CRF.

It is generally agreed that prothrombin occupies the key position in physiological blood clotting.[18] Coagulase, accordingly, might replace thromboplastin, stable and labile accelerators, calcium, and the like, and "activate" the prothrombin molecule directly to effect the conversion of fibrinogen to fibrin. In addition to a partial correspondence of CRF and prothrombin activity in similar globulin fractions, other considerations lent weight to the possibility that CRF and prothrombin might have a close relationship to each other or might even be identical.[2, 26] Both substances have many properties in common, such as similar zones of heat inactivation, and both are similarly absorbed by such substances as barium sulfate, aluminum hydroxide, and magnesium hydroxide. When blood is coagulated by the physiological mechanisms involving the conversion of prothrombin to thrombin, prothrombin and CRF are consumed at the same rate.[30] Similarly, rabbits treated with the anticoagulants dicoumarol[2, 32] and phenylindanedione[32] lose prothrombin and CRF at an almost equal pace, and the reversal of the anticoagulant effect by vitamin K_1 affects the restoration of both factors equally. It is also of interest that in a refractory rabbit both prothrombin and CRF were unaffected by the anticoagulants.[32]

TABLE 2

PURIFICATION OF CRF

(1) Filter plasma 5 times through Seitz sterilizing asbestos pads.
(2) Pack column with celite (hyflo) and alumina hydroxide gel (amphogel), using 250 grams of hyflo and 55 ml. of amphogel per liter of plasma. Process all column samples by vacuum-pump suction.
(3) Absorb 1000 ml. of plasma on column. Discard the eluate.
(4) Add 1000 ml. of 0.1 M phosphate buffer, *p*H 8 to column. Discard eluate.
(5) Add 500 ml. of 0.2 M phosphate buffer, *p*H 8 to the column. Discard eluate.
(6) Add in 250 ml. lots 1000 ml. of 0.2 M phosphate buffer, *p*H 8.6. Save and test all fractions for activity. Adjust *p*H to 7.0.
(7) To the most active fraction of No. 6, add ammonium sulfate crystals to 33 per cent saturation. Incubate at 0° C. for 6 to 12 hours, centrifuge precipitate, and discard it.
(8) To the supernatant solution of fraction 7, add ammonium sulfate crystals to 45 to 50 per cent saturation. Incubate at 0° C. for 6 to 12 hours, collect precipitate, and discard supernatant solution.
(9) Dissolve precipitate in saline. Store in the deep freeze at −20° C.

Both physiological and coagulase clotting are potentiated by minute amounts of crystalline trypsin.[31]

While all of these considerations are highly suggestive, they do not constitute valid proof that CRF and prothrombin are the same compound. It became apparent that the problem might only be resolved by 2 lines of attack: (1) a significant separation of prothrombin and CRF activity; and (2) the purification and characterization of CRF and of prothrombin as distinct substances.

The dissociation of CRF and prothrombin activity was successfully accomplished.[26] When human citrated plasmas were filtered 5 times through sterilizing asbestos Seitz pads, as much as 50 per cent of CRF activity was recovered in the filtrates, while prothrombin activity was virtually eliminated. To demonstrate such dissociation, careful attention must be paid to the surface-volume relationships of the filtration procedure.[29] Using such filtrates as the starting point, concentration and purification studies were undertaken. A representative scheme is summarized in TABLE 2.[33]

The purified CRF was studied in the ultracentrifuge and on paper electrophoresis.[34] It was found that the CRF preparation formed a homogeneous symmetrical peak, with sedimentation rates in the range of 2.5 to 3, constituting about 90 per cent of the material, but a small rapidly moving component with a sedimentation rate of 5.85 could not be eliminated. On paper electrophoresis, the maximal staining was in the zone between the beta and the gamma globulins. Neither the molecular weight nor the behavior on paper electrophoresis corresponded to the reported properties of highly purified bovine prothrombin of W. H. Seegers.[7] Thus, the CRF obtained upon purification following Seitz filtration differs not only in activity from prothrombin but also in certain basic properties, such as molecular weight and mobility on paper electrophoresis.

If, then, "pure" prothrombin were available and found to be free of CRF activity, the case for the distinctiveness of CRF from prothrombin would be fully established. Earlier tests of the purified bovine prothrombin of Seegers showed these preparations to be devoid of CRF,[30] but these tests are not perti-

nent to the present problem since bovine plasma is itself very low in CRF activity. It was therefore most fortunate that we were able to secure highly purified human prothrombin from Seegers. This substance was prepared in Seegers' laboratory by Takeshi Abe from citrated human plasmas shipped in a solid-frozen state from Atlanta, Ga. to Detroit, Mich. The products were tested in the ultracentrifuge, by paper electrophoresis, and for biological activity. In the ultracentrifuge under varying conditions, sedimentation rates differing from those obtained with CRF were obtained 4.5 and 5.6 were obtained for the principal component while, with paper electrophoresis, the protein stain was localized primarily in the a_1 and a_2 positions. Though clearly different from the purified CRF in its physical properties, the purified prothrombin proved to be an excellent source of CRF. Indeed, the purified prothrombin preparations were equally as potent as some of the most active CRF samples prepared from Seitz-filtered plasmas which, nevertheless, were devoid of prothrombin activity.

A synthesis and reconciliation of these findings is certainly necessary. The many points of similarity between prothrombin and CRF have already been summarized. Certain established differences between the physiological and the coagulase clotting systems do not necessarily involve the question of the identity of CRF and prothrombin. Thus the clotting of plasma by staphylocoagulase in the presence of citrate and oxalate ions and of heparin may merely indicate that the mode of attack on the prothrombin molecule by the bacterial product differs from the physiological activation. Certain differences do point to a separation of the 2 factors, however. Discrepancies have been noted in CRF and prothrombin activity in different plasma fractions.[25] There is also the well-established dissociation of CRF and prothrombin activity in different animal species. The most unequivocal separation of CRF and prothrombin was accomplished by the serial Seitz filtration procedure already described.

The demonstration of CRF activity in a purified plasma fraction distinct from prothrombin, and also in a highly purified prothrombin preparation, suggests several possible interpretations:

(1) This activity may be a fortuitous occurrence, and the 2 substances may be wholly unrelated.

(2) The fractions may be contaminated. Admittedly, neither preparation is completely homogeneous, although the major component of each is symmetrical and constitutes well over 90 per cent of the total material in each instance. When activity was tested after paper electrophoresis in parallel unstained strips, however, it was found to correspond very closely to the area of maximum staining. Similarly, the use of a separation cell in a Spinco analytical ultracentrifuge pointed to an association of activity with the major component.

(3) Purified CRF recovered after Seitz filtration and prothrombin may be related compounds. Prothrombin may be the parent compound, but it may break up into smaller units that no longer function as prothrombin but still function as CRF. Some of these fractions may be intermediate products between prothrombin and thrombin and, indeed, human thrombin[26] itself is still effective as CRF, once dilutions are carried beyond the zone of reactivity with fibrinogen alone.

A number of considerations lead us to favor the third possibility:

(1) The purified CRF prepared from Seitz-filtered plasma has shown a principal component with a molecular weight estimated at one half that of one of the prothrombin components as determined in the Spinco ultracentrifuge.

(2) The properties of CRF obtained after filtration differ sharply from those of CRF in untreated plasma.[29] Thus precipitability by the Mellanby dilution and acidification technique, adsorption on alumina, heat stability, and heparin sensitivity are all significantly altered by filtration.

(3) The deliberate conversion of purified Seegers' prothrombin to a derivative, autoprothrombin, failed to destroy CRF activity although it had eliminated prothrombin activity and had a low thrombin content. This preparation, when studied in the ultracentrifuge, showed a disruption of the prothrombin molecule into many smaller components.

(4) It has been noted that when a plasma low in CRF is treated chemically, as by the Mellanby precipitation technique, an unexpected increase in CRF activity is noted in the reconstituted precipitate.[26] This has been previously interpreted as a phenomenon of release from inhibitors. In the light of the current hypothesis, it may be that the chemical procedure has broken up or modified a larger, less reactive prothrombin molecule into smaller units that function more effectively as CRF.

(5) It has been shown that crystalline soybean trypsin inhibitor (SBTI) fails to block the coagulase clotting of plasma.[31] It has been established that SBTI fails to block the reaction between thrombin and fibrinogen,[14, 34] but is effective in blocking the conversion of prothrombin. If active CRF includes fractions intermediate between prothrombin and thrombin, it is now understandable why this inhibitor failed to block the coagulase clotting of plasma.

In the light of the studies presented, based on highly purified though regrettably not homogeneous components, it is now possible to reconcile the conflicting findings on the nature of CRF. Given a susceptible animal species, coagulase can react with (1) prothrombin, (2) smaller molecules presumably derived from prothrombin, and (3) thrombin itself. It is now understandable why CRF and prothrombin activity parallel each other very closely while, in other instances, a dissociation of activity occurs. The CRF obtained after Seitz filtration permits the isolation of small molecules intermediate between prothrombin and thrombin. It is uncertain whether these substances are normal components of plasma or are formed as a result of the filtration.

How coagulase acts is still unknown. Since prothrombin may be activated by trypsin and other proteolytic enzymes, it was deemed possible that coagulase might function similarly. No direct evidence of proteolysis has been found, however. Coagulase is not inhibited by soybean trypsin inhibitor,[31] and there is no obvious action on such substrates as fibrin, gelatin, and egg white. Sol Sherry[30] has been unable to find any evidence of the splitting of synthetic arginine and lysine esters by coagulase, a delicate test for proteolysis. The mechanism of coagulase action may therefore be of some interest to the worker on the physiological clotting of blood since it presents an efficient, if unusual, approach to analyzing prothrombin and its derivatives. It is also noteworthy that the no man's land between prothrombin and thrombin is coming under scrutiny in relation to blood clotting factors of significance in physiological

clotting.[6] It has been recently suggested, accordingly, that plasma thromboplastin component (PTC) may be a derivative of prothrombin that serves as a ready source of thrombin.[22] Possibly other prothrombin conversion accelerators may be shown to have a close relationship to prothrombin, or even to be derived from it. Comparative studies in our laboratory of CRF and platelet suspensions, the serum prothrombin conversion accelerator (SPCA) of Alexander, the labile accelerator globulin, and PTC have failed to indicate a correspondence of properties. We have been unable to support the claim that CRF is identical with the thromboplastinogen or the antihemophilic globulin.[12] Further work along these lines is indicated, however.

It has generally been assumed that after coagulase reacts with its substrate, CRF, fibrinogen is converted to fibrin. It was therefore of interest to study the fibers under the electron microscope. Preliminary observations were made with Albert Lansing on "fibrin" formed from the reaction of purified coagulase, CRF, and human fibrinogen. Such clots were fixed in osmic acid and then sectioned. While sufficient fibers were not examined to justify any statistical comparison with fibrinogen, fibers showing the same range of periodicity as fibrinogen were observed, as well as some fibers with much coarser striations.

While this report has been primarily concerned with defining the factors that lead to the clotting of plasma by staphylocoagulase, other studies have directly implicated coagulase in staphylococcal virulence. Smith, Hale, and Smith[24] have proposed that the *in vivo* elaboration of coagulase delays the phagocytosis of staphylococci by its ability to initiate the deposition of fibrin. Alternative biological functions for coagulase have been suggested, such as the enhancement of the survival of staphylococci in leukocytes,[17] and the ability of coagulase to block the antibacterial activity of normal human serum.[7] It is evident from other contributions to this monograph that the mechanisms of staphylococcal virulence in general, and the contribution of coagulase to virulence in particular, remain both complex and elusive. If an *in vivo* role is to be ascribed to coagulase, then it might be on the basis of its clotting action or on the basis of some as yet undefined attack on host tissue acting as the substrate. The determination of the enzymatic activity of purified coagulase, therefore, may prove of interest in both the blood-clotting field and in an analysis of staphylococcal virulence. Studies along these lines are in progress.

Summary

The properties of highly purified staphylocoagulase are presented. The evidence for the identification of the coagulase-reacting factor of plasma is discussed. On the basis of studies with highly purified preparations it is concluded that CRF activity is linked with the prothrombin molecule, but that smaller molecules, devoid of prothrombin activity but presumably derived from prothrombin, are also effective in reacting with coagulase.

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THE EFFECT OF COAGULASE ON THE ANTIBACTERIAL ACTIVITY OF NORMAL HUMAN SERUM AGAINST SELECTED STRAINS OF *MICROCOCCUS PYOGENES*

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Although coagulase production has been used for many years as a criterion for the potential pathogenicity of staphylococcal strains isolated from infectious processes, the actual mechanism of its action in the pathogenesis of staphylococcal infections is still enigmatic. That coagulase does play a role in the pathogenesis of infection with these organisms was indicated by the work of Smith and Hale (1944) who showed that the correlation between coagulase production and pathogenicity holds only when the plasma of the animal concerned is coagulable.

Inhibition of phagocytosis by coagulase in an *in vitro* system was advanced as one explanation of the role of coagulase in staphylococcal infections by Hale and Smith (1945). That coagulase functions *in vivo* as it does *in vitro* has been questioned, however, since it has been shown that even with the injection of massive amounts of potent cultures and filtrates into rabbits, no intravascular clotting could be demonstrated clinically, grossly, or microscopically (Fisher, 1936). Menkin and Walston (1935) were also unable to produce lymphatic blockage in rabbits by the intracutaneous injection of active cell-free coagulase.

The present work was undertaken with the thought of developing a more direct test of virulence for the staphylococci. Since all of the *in vitro* tests commonly used take into consideration only certain biochemical activities of the organisms, many of which have not been fundamentally associated with virulence, their reliability is always open to some criticism. It was felt that a more direct test of virulence that would depend not only upon the activities of the organisms but also upon the relative resistance of the host to the organism in question would be of definite value.

The investigation reported here includes a comparison of the growth of pathogenic and nonpathogenic strains of staphylococci in normal human serum, and the effect of various metabolic products of the microorganisms upon this growth.

Previous work (Hench, 1952) had indicated that the susceptibility of an animal to an infectious agent was reflected in the ability of that animal's serum to support the growth of the organism in question. With this thought in mind, 35 strains of staphylococci were tested for their ability to grow in pooled human serum containing 20 individual serum samples.

The strains tested included not only recently isolated strains from human sources but also laboratory strains and strains of animal origin. The preliminary screening was done by inoculating 0.1 ml. of a 10^{-6} dilution of an 18-hour broth culture of the organism under examination into 1.0 ml. of the pooled serum. This inoculum contained an average of 2.0×10^2 organisms. A 0.1 ml. aliquot was removed after 24 hours incubation at 37° C. and plated directly. The tests were carried out in serological tubes 10 mm. × 75 (outside dimen-

sions) that were kept closed with rubber stoppers during incubation. This method prevented the rapid alkaline shift of the serum that occurs when serum is exposed to the air without addition of further buffers. The organisms were also tested by accepted methods for coagulase production, mannitol fermentation, pigmentation, and hemolysis on rabbit- and sheep-blood agar plates. The results of this screening are presented in TABLE 1, where the close correlation between coagulase production and growth in serum is evident. Scoring the growth of the organisms in serum was done on an arbitrary numerical basis. In TABLE 1, the number 4 indicates confluent growth on the

TABLE 1

SURVEY OF STRAINS OF *MICROCOCCUS PYOGENES* WITH RESPECT TO PHYSIOLOGICAL PROPERTIES AND GROWTH IN NORMAL HUMAN SERUM

Strain	Coagulase	Pigment	Mannitol fermentation	Toxins		Growth in serum
				α	β	
Recent isolates human origin						
Kurtz	+	+	−	−	−	4
McKelton	+	+	+	+	+	4
Colby	+	+	+	−	−	4
Wells	+	+	+	+	−	4
Wilker	+	+	+	−	−	4
Schultz	+	+	+	+	−	4
Feastman	+	+	+	+	−	4
Pridmore	+	+	+	+	−	4
Sloenellby	+	+	+	+	+	4
Nichols	+	+	+	+	−	4
V.A.	+	+	+	+	−	4
Morgan	+	+	+	+	−	4
Slocum	+	+	+	+	−	4
Houch	−	−	−	−	−	0
Hill	−	−	−	−	−	0
Bates	±	+	+	−	−	0
Barile	−	−	−	−	−	0
Ekstedt	−	−	−	−	−	0
Lofgren	−	−	−	−	−	0
Fisher	−	−	−	−	−	0
Callahan	−	−	−	−	−	0
Ganley	−	−	−	−	−	1
Coultas						0
van H.	±	+	−	−	−	1
Animal strains						
VS 346	±	+	+	−	−	0
J-32-A	±	+	+	−	+	1
VS 393	+	+	+	+	+	2
Staph. 24	+	−	+	+	+	0
Laboratory strains						
196 E	+	+	+	+	+	4
van	+	+	+	+	−	4
P 209	+	+	+	+	−	4
Pen. resis.	+	+	+	+	−	4
Type A	+	+	+	+	−	4
Type B	−	−	−	−	−	1
albus AP	−	−	−	−	−	0

Table 2

Growth of Serum-Susceptible and Serum-Resistant Strains of *Micrococcus pyogenes* in Normal Human Serum and Broth

Time hrs.)	Resistant strain in serum	Susceptible strain in serum	Resistant strain in broth	Susceptible strain in broth
0	2.0×10^{1}*	4.8×10^{2}	4.0×10^{2}	4.0×10^{2}
1	5.0×10^{1}	5.3×10^{2}	7.0×10^{2}	4.0×10^{2}
3	2.2×10^{2}	8.5×10^{2}	6.0×10^{3}	6.6×10^{3}
5	4.7×10^{4}	3.5×10^{3}	5.2×10^{5}	3.5×10^{4}
10	1.1×10^{7}	4.0×10^{3}	2.2×10^{8}	1.3×10^{7}
24	6.0×10^{7}	4.0×10^{3}	—	—

* Plate count per 1.0 ml. serum.

plates from the 24-hour sampling, 1 indicates approximately the same number of colonies in the 0 and 24 hour sampling, and 0 indicates fewer colonies developed after 24 hours in serum than were seen on plates made at zero time.

In all succeeding experiments, actual counts were made of the number of surviving organisms after varying intervals of exposure to serum. The inoculum in most cases was kept low, that is, of the order of 10^2 to 10^3 organisms per ml., in order to simulate as closely as possible the conditions that would be likely to occur in the initiation of an infection.

The results of a typical experiment comparing the growth of coagulase-positive serum-resistant and coagulase-negative serum-susceptible strains of *Staphylococcus* in serum and broth are presented in TABLE 2.

With the difference in the susceptibility of nonpathogenic coagulase-negative and pathogenic coagulase-positive strains of staphylococci to the effects of normal human serum noted, the problem was approached from 2 points of view. First, an attempt was made to learn more about the antistaphylococcal serum factor and, second, we studied the serum-resistant organisms to discover how these strains were capable of withstanding the inhibitory effects of serum.

Heat Stability

The first experiments to determine the characteristics of the antibacterial serum factor were designed to study its heat stability. Two ml. samples of undiluted serum were sealed in soft glass tubes to prevent evaporation and heated in a water bath at 55° C. or 60° C. for 30 minutes. An unheated sample of the same serum served as the control. The results of this experiment are presented in TABLE 3. It is apparent that the serum activity is heat stable in

Table 3

Growth of a Serum-Susceptible Strain of *Micrococcus pyogenes* in Heated Normal Human Serum

Time (hrs.)	Unheated serum	Serum heated 56° C./30 min.	Serum heated 60° C./30 min
0	2.1×10^{3}*	2.4×10^{3}	2.0×10^{3}
10	4.0×10^{4}	2.4×10^{3}	2.1×10^{3}
24	5.0×10^{4}	2.7×10^{4}	3.6×10^{3}

* Plate count per 1.0 ml. serum.

TABLE 4

GROWTH OF VARIED INOCULA OF A SERUM-SUSCEPTIBLE STRAIN OF *MICROCOCCUS PYOGENES* IN HEATED NORMAL HUMAN SERUM

Time (hrs)	Dilution†			
	10^{-3}	10^{-4}	10^{-5}	10^{-6}
	Unheated Serum			
0	2.9×10^{4}*	3.6×10^{3}	6.2×10^{2}	1.5×10^{2}
10	5.4×10^{3}	1.5×10^{2}	3.0×10^{1}	0
24	5.0×10^{3}	2.0×10^{2}	2.0×10^{1}	0
	Heated at 56° C./30 min.			
0	2.9×10^{4}	4.2×10^{3}	5.9×10^{2}	4.0×10^{1}
10	1.2×10^{2}	7.0×10^{1}	2.0×10^{1}	0
24	4.5×10^{1}	5.0×10^{1}	1.8×10^{1}	0
	Heated at 60° C./30 min.			
0	3.3×10^{4}	5.4×10^{3}	6.6×10^{2}	9.0×10^{1}
10	7.2×10^{5}	1.3×10^{5}	1.5×10^{3}	5.0×10^{2}
24	5.2×10^{7}	3.4×10^{6}	5.5×10^{3}	4.5×10^{2}

* Plate count per 1.0 ml. serum.
† Dilution of 18-hour broth culture of organisms in saline.

the usual sense of the word for biological materials, that is, at 56° C. for 30 minutes. Under the conditions of this experiment it appeared to be capable also of withstanding temperatures as high as 60° C. for 30 minutes. With the small inocula used in this experiment, any slight impairment of the antibacterial activity might have been missed. A further heating experiment was therefore carried out in which varying inocula were introduced into heated sera. The results are presented in TABLE 4. From these results it appears that the anti-staphylococcal activity of human serum is stable at 56° C. but shows signs of inactivation at 60° C. Heating undiluted serum above 62° C. results in its coagulation.

Nutritional Requirements

The possibility that the differential antibacterial effect upon the different strains of staphylococci was due to differences in nutritional requirements of the organisms was next investigated. It was felt that the best way to test this possibility was to add a complete nutrient medium to serum in increasing amounts and to record the subsequent growth of an otherwise serum-susceptible strain in this nutritionally fortified serum. As a control, 1 per cent gelatin in saline was identically fortified with nutriment, and the growth of identical inocula was followed. The results of this experiment are presented in TABLE 5. It was only after the serum had been diluted with more than an equal volume of brain-heart infusion (BHI) broth (the nutrient used) that there was any appreciable growth. Even in this case the growth did not exceed that in the control tube, into which only 0.1 ml. of BHI was placed.

TABLE 5
GROWTH OF A SERUM SUSCEPTIBLE STRAIN OF *MYCROCOCCUS PYOGENES* IN SERUM-BROTH AND GELATIN IN SALINE-BROTH MIXTURES

Time (hrs.)	Serum-Broth Mixtures						
	1.0/0.0*	0.9/0.1	0.8/0.2	0.7/0.3	0.6/0.4	0.5/0.5	0.4/0.6
0	5.1×10^2	4.8×10^2	4.7×10^2	6.1×10^2	3.9×10^2	4.8×10^2	3.5×10^2
6	2.7×10^3	6.0×10^3	3.0×10^3	1.2×10^3	3.4×10^3	4.8×10^3	1.5×10^5
24	1.8×10^3	5.7×10^3	9.6×10^3	1.4×10^4	3.0×10^4	3.6×10^5	3.0×10^7

	Gelatin in Saline-Broth Mixtures						
	1.0/0.0†	0.9/0.1	0.8/0.2	0.7/0.3	0.6/0.4	0.5/0.5	0.4/0.6
0	1.2×10^3	1.5×10^3	1.3×10^3	1.3×10^3	1.5×10^3	1.2×10^3	1.3×10^3
6	0	3.6×10^4	6.0×10^4	3.6×10^4	7.2×10^4	9.0×10^4	7.8×10^4
24	0	3.5×10^8	3.6×10^8	4.8×10^8	5.8×10^8	9.6×10^8	9.0×10^8

* Dilution: 1.0 ml. serum per 0.0 ml. broth, *etc.*
† Dilution: 1.0 ml. gelatin in saline per 0.0 ml. broth, *etc.*

Calcium Requirements of the Reaction

Jacox (1950), studying the bactericidal effect of human serum on *Bacillus subtilis*, demonstrated that the effect was calcium sensitive and could be abolished by treating the serum in such a way as to make the calcium unavailable.

Similar experiments were carried out with the serum-susceptible strains of staphylococci. Sterile 0.1 M solutions of sodium citrate and calcium chloride were prepared. To 1.0 ml. serum samples were added 0.1 ml. amounts of sodium citrate and calcium chloride solutions, separately and in equivalent amounts. Volumes were kept constant in all tubes by the addition of sterile water. These treated sera and control sera, diluted identically with water, were tested for their ability to support the growth of serum-susceptible strains of staphylococci. The results of a typical experiment of this kind are presented in TABLE 6. The antistaphylococcal activity was considerably reduced in the tubes receiving only the citrate but, upon addition of an equivalent amount of calcium, the antistaphylococcal activity was reestablished. In

TABLE 6
EFFECT OF ADDITION OF SODIUM CITRATE AND EQUIVALENT AMOUNTS OF CALCIUM CHLORIDE UPON THE ANTIBACTERIAL ACTIVITY OF NORMAL HUMAN SERUM AGAINST A SUSCEPTIBLE STRAIN OF *MYCROCOCCUS PYOGENES*

Time (hrs.)	Control	Na Citrate*	Na Citrate + equiv. $CaCl_2$	$CaCl_2$*
0	0.6×10^2†	4.3×10^2	4.5×10^2	4.1×10^2
3		3.6×10^2	5.2×10^2	3.0×10^2
6	9.4×10^2	7.2×10^2	1.4×10^3	3.0×10^2
10	2.6×10^3	5.1×10^3	3.3×10^3	1.6×10^2
24	8.5×10^2	4.8×10^6	1.4×10^3	1.8×10^2

* 0.1 M solutions added in 0.1 ml. amounts to 1.0 ml. of serum.
† Plate count per 1.0 ml. serum.

addition we found, in agreement with Jacox (1950), that the Ca^{++} could not be replaced in the reaction by Mg^{++}, Mn^{++}, or Fe^{++} ions.

Oxidation-Reduction Studies

Hench (1952) found that an oxidation-reduction mechanism played a role in the pneumococcidal activity of rat and guinea pig serum. The serum from the guinea pig, an animal relatively resistant to experimental pneumococcal infection, was highly bactericidal for the pneumococcus, while serum from the susceptible rat lacked this activity. Hench found, however, that by the addition of reducing agents the pneumococcidal activity of the guinea pig serum could be appreciably reduced. Pichat (1949) also noted that the bactericidal activity of blood, serum, and urine toward staphylococci, typhoid, paratyphoid, and colon bacilli could be completely abolished by the intravenous injection of 3 gm. of neutralized ascorbic acid.

In view of these findings, experiments were undertaken to determine the effect of certain reducing agents upon the antibacterial activity of normal human serum against susceptible staphylococci *in vitro*.

Sodium ascorbate was prepared at a concentration of 40 mg. per ml. and sterilized by filtration through a Seitz filter. Doubling dilutions of this stock solution were made, and 0.1 ml. amounts of the solutions were added to 1.0 ml. of serum. Additions were equivalent to 4, 2, 1, 0.5, 0.25, and 0.0 mg. of sodium ascorbate per ml. of serum. The pH and Eh of the tubes before the experiment were determined on a control series of tubes set up exactly as were the experimental tubes. This was necessary to preserve the sterility of the experimental tubes. These determinations after the experiment were made upon the actual experimental tubes to detect any change due to the growing organisms. The pH and Eh determinations were made with a Beckman Model G pH meter using a Dietz (1949) microcup.

The results of a typical experiment of this kind are shown in TABLE 7. Only in the tubes where the reducing agent was at a sufficient concentration to maintain the Eh values below -0.05 volts was there any appreciable growth of the otherwise susceptible strains. Similar results were obtained using sodium thioglycollate as the reducing agent.

Proteolytic Digestion of Serum

The possibility that the antibacterial activity of the serum was associated with a protein or protein complex was investigated by treating serum with pepsin and then testing its antistaphylococcal activity against susceptible staphylococci. Serum was adjusted to pH 4.5 with 5 N HCl, 10 mg. per ml. of granular pepsin (Lilly, 1:10,000) was added and mixed thoroughly, and the tubes were incubated at 30° C. Samples were taken at 1 and 5 hours, and adjusted to pH 7.5 with 5 N NaOH, which effectively stopped the enzymatic activity. The samples were then dispensed for the antibacterial test. Controls were included to determine the effect of mere pH adjustment to pH 4.5 on the antibacterial activity, and the effect of the added pepsin without pH adjustment was also considered. The tubes of digested serum were then inoculated with a suscep-

TABLE 7

THE EFFECT OF ASCORBIC ACID UPON THE ANTIBACTERIAL ACTIVITY OF NORMAL HUMAN SERUM AGAINST A SERUM-SUSCEPTIBLE STRAIN OF *MYCROCOCCUS PYOGENES*

Time (hrs.)	Ascorbic Acid					
	4 mg.*	2 mg.	1 mg.	0.5 mg.	0.25 mg.	0.0 mg.
0	2.0×10^2†	2.6×10^2	2.5×10^2	1.8×10^2	1.8×10^2	2.6×10^2
24	9.0×10^5	3.0×10^4	1.5×10^4	1.3×10^2	1.8×10^2	2.4×10^2
	Before experiment					
pH...	7.3	7.5	7.5	7.6	7.7	8.0
Eh....	−0.085v	−0.092	−0.085	−0.079	−0.070	+0.03
	After experiment					
pH...	6.7	7.5	7.2	7.4	7.3	7.5
Eh....	−0.07v	−0.11	−0.05	+0.01	+0.11	+0.10

* Mg. neutralized ascorbic acid per 1.0 ml. serum.
† Plate counts per 1.0 ml. serum.

tible strain of *Staphylococcus*, and its growth was followed. The results of these experiments are presented in TABLE 8. They clearly show that the antibacterial activity of normal human serum for staphylococci can be reduced by proteolytic digestion with pepsin.

Absorption Studies

Absorption with heat-killed whole bacterial cells. It was obviously important in a study of this kind to determine whether the antibacterial activity shown by normal serum against certain strains of staphylococci was of a specific nature or merely a manifestation of a more general activity.

Agglutination experiments were performed early in this investigation to determine whether the consistently lower counts obtained with the serum-susceptible staphylococci were valid population estimates or due solely to aggregation of the susceptible strains. At no time could aggregates larger than those present in the controls be observed either microscopically or macroscopically.

Absorption experiments were carried out in which serum samples were ab-

TABLE 8

THE EFFECT OF PEPSIN DIGESTION OF NORMAL HUMAN SERUM UPON ITS ANTIBACTERIAL ACTIVITY AGAINST A SERUM-SUSCEPTIBLE STRAIN OF *MYCROCOCCUS PYOGENES*

Time (hrs.)	pH control	Enzyme control	1-hour digestion	5-hours digestion
0	1.1×10^3*	1.5×10^3	1.3×10^3	1.1×10^3
5	6.0×10^2	3.0×10^2	9.2×10^2	7.1×10^1
10	1.2×10^3	4.5×10^3	6.5×10^3	4.4×10^2

* Plate count per 1.0 ml. serum.

sorbed with susceptible and resistant strains of staphylococci and then tested for their ability to support the growth of these organisms. Absorptions were also carried out with other organisms including: *Streptococcus pyogenes*, group A; *Diplococcus pneumoniae*, type I, strain 76; *Salmonella typhosa*, strain Rawlings; and *Bacillus anthracis*, strain H 109. The details of the absorption experiments have been presented elsewhere (Ekstedt, 1956). A composite table of the absorption results with whole cells are presented in TABLE 9 and indicate that the antistaphylococcal activity of normal human serum is not of the usual serological specificity since the activity can be reduced by both serum-susceptible and serum-resistant staphylococci as well as other unrelated organisms.

Absorption with cellular and filtrate fractions. Fractionation of both susceptible and resistant staphylococcal strains was carried out in an attempt to determine if there was a particular cellular element responsible for the different biological activity observed. Fractions were also prepared from culture filtrates and tested for their ability to neutralize the antistaphylococcal activity of serum for susceptible strains.

Polysaccharide fractions, prepared according to the method of Julianelle and Wieghard (1935) from both serum-susceptible and serum-resistant staphylococci were used to treat serum. These fractions from neither serum-susceptible nor serum-resistant strains of staphylococci neutralized the antibacterial activity of the serum against susceptible strains.

Purified alpha and beta toxins were prepared by acetone precipitation at pH 4.0 and 9.0 respectively, according to a method described by Fulton (1943). These preparations, when used to treat serum, were without effect upon its antistaphylococcal activity.

Crude protein fractions were prepared from ruptured-cell supernatant by saturation with ammonium sulfate. Even with repeated washing of the coagulase-positive, serum-resistant strains of staphylococci, the product almost always had coagulase activity. These crude protein fractions, prepared identically from both serum-susceptible and serum-resistant strains of staphylococci, were used to treat active serum in amounts of 1.0 mg. protein per 1.0 ml. serum.

TABLE 9

GROWTH OF A SERUM-SUSCEPTIBLE STRAIN OF *MYCROCOCCUS PYOGENES* IN NORMAL HUMAN SERUM ABSORBED WITH HOMOLOGOUS AND HETEROLOGOUS ORGANISMS

Time (hrs.)	Absorbent		
	Susceptible Staphylococci	Resistant Staphyloccoci	Streptococci
0	6.5×10^{2}*	5.0×10^{2}	6.0×10^{2}
24	9.0×10^{7}	1.2×10^{8}	1.0×10^{6}
	Pneumococci	Typhoid	Anthrax
0	8.0×10^{2}	6.4×10^{2}	6.8×10^{2}
24	1.0×10^{7}	2.4×10^{3}	2.0×10^{3}

* Plate count per 1.0 ml. serum.

TABLE 10

GROWTH OF A SERUM-SUSCEPTIBLE STRAIN OF *MYCROCOCCUS PYOGENES* IN NORMAL HUMAN SERUM TREATED WITH THE CELL PROTEINS DERIVED FROM SUSCEPTIBLE AND RESISTANT STRAINS OF THESE ORGANISMS

Time hrs	Control serum	Serum treated with susc. cell protein	Serum treated with resist. cell protein
0	6.2×10^{2}*	7.2×10^{2}	7.5×10^{2}
5	1.2×10^{3}	7.2×10^{2}	1.0×10^{3}
10	1.1×10^{3}	7.0×10^{2}	1.5×10^{5}
24	6.2×10^{2}	6.4×10^{1}	3.9×10^{7}

* Plate counts per 1.0 ml. serum.

The results of a typical experiment with serum treated with the cellular proteins from susceptible and resistant strains is shown in TABLE 10. Serum treated with resistant-strain protein was rendered inactive against a serum-susceptible strain, while serum treated identically with serum-susceptible-strain protein was still antibacterial against serum-susceptible organisms. Protein fractions similarly prepared from filtrates of the organisms also gave similar results (TABLE 11). The filtrate protein fraction in this instance had a coagulase titer such that 10 μg. of the material produced a solid clot in human plasma diluted 1:5 in less than 1 hour. The activity of this material in neutralizing the antistaphylococcal substances in human serum directed against certain strains of staphylococci was stable to heating at 65° C. for 30 minutes. This treatment had no effect on the coagulase titer.

An interesting observation was made upon 2 strains of staphylococci that were very weak coagulase producers. These strains were nevertheless susceptible to the antistaphylococcal activity of human serum. Upon further fractionation of the crude preparations resulting from saturation of culture filtrate with ammonium sulfate, preparations were obtained that had low coagulase activity. These preparations were incapable of neutralizing the antistaphylococcal activity of serum to coagulase-negative strains. When used to treat serum into which was inoculated the weak coagulase producers, however, these organisms would grow. The results of 1 of these experiments are presented in TABLE 12. It seems from these, as well as from other experiments, that a min-

TABLE 11

THE EFFECT OF AMMONIUM SULFATE PRECIPITATED CULTURE FILTRATE MATERIAL FROM SERUM-SUSCEPTIBLE AND SERUM-RESISTANT STRAINS OF *MICROCOCCUS PYOGENES* UPON THE GROWTH OF SUSCEPTIBLE STRAINS IN NORMAL HUMAN SERUM

Time (hrs.)	Untreated serum control	Serum treated with resistant strain filtrate material*			Serum treated with susceptible strain filtrate material		
		Strain no. 1.	Strain no. 2.	Strain no. 3.	Strain no. 1.	Strain no. 2.	Strain no. 3.
0	6.5×10^{2}†	1.3×10^{2}	5.0×10^{1}	3.0×10^{2}	1.5×10^{2}	5.0×10^{1}	5.0×10^{1}
10	4.2×10^{2}	5.0×10^{5}	3.5×10^{5}	2.0×10^{5}	1.2×10^{2}	3.5×10^{1}	5.0×10^{1}
24	2.3×10^{2}	5.0×10^{7}	8.0×10^{6}	1.8×10^{7}	1.2×10^{2}	4.0×10^{1}	2.0×10^{1}

* 1.0 mg. of ammonium sulfate saturated culture-filtrate precipitate per 1.0 ml. of serum.
† Plate counts per 1.0 ml. serum.

TABLE 12

GROWTH OF WEAKLY COAGULASE-POSITIVE AND COAGULASE-NEGATIVE STRAINS OF *MICROCOCCUS PYOGENES* IN NORMAL HUMAN SERUM TREATED WITH A WEAKLY-ACTIVE COAGULASE PREPARATION

Time (hrs.)	Weakly coagulase-positive strain in treated serum*	Coagulase-negative strain in treated serum	Coagulase-negative strain in untreated serum
0	6.4×10^2†	1.5×10^2	2.1×10^2
10	4.5×10^5	2.0×10^2	3.0×10^2
24	3.0×10^7	3.0×10^3	3.0×10^3

* Serum treated with a weakly active coagulase preparation.
† Plate counts per 1.0 ml. serum.

imum amount of active coagulase is necessary to reverse the antistaphylococcal activity of serum.

Further Study of the Protective Material

Further fractionation of the saturated ammonium sulfate preparations from coagulase-positive resistant-strain filtrates was carried out. The crude preparation was dissolved at a concentration of 1.0 per cent in distilled water, chilled to 0° C. in an ice bath, and solid ammonium sulfate calculated to raise the salt concentration to one third saturation added slowly with constant agitation. The resulting precipitate was separated in a refrigerated centrifuge and the supernatant fluid raised to two thirds saturation by the further addition of solid ammonium sulfate. The precipitates and the final supernatant were redissolved in distilled water, dialyzed free from salts and dried by lyophilization. Tubes containing 1.0 mg. of the dried fractions per ml. of serum were prepared. Susceptible strains of staphylococci were inoculated into the serum thus treated, and their growth followed over a 24-hour period. The fractions were also ti-

TABLE 13

GROWTH OF SERUM-SUSCEPTIBLE STRAINS OF *MICROCOCCUS PYOGENES* IN NORMAL HUMAN SERUM TREATED WITH AMMONIUM SULFATE FRACTIONS OF RESISTANT STRAINS OF THESE ORGANISMS

	Time (hrs.)	Coagulase titer		
		Negative	1:160	1:5120
		$(NH_4)_2SO_4$ fract.		
		⅓ sat.	⅔ sat.	full sat.
Strain no. 1.*	0	7.0×10^2†	7.2×10^2	6.5×10^2
	24	5.0×10^3	2.4×10^7	1.2×10^7
Strain no. 2.	0	6.4×10^1	6.0×10^1	6.0×10^1
	24	3.0×10^2	8.0×10^2	9.0×10^6
Strain no. 3.	0	2.0×10^2	1.9×10^2	2.6×10^2
	24	2.4×10^2	1.0×10^2	6.0×10^6

* Strain no. 1 was a weakly coagulase-positive strain, still susceptible to serum bacteriostasis. Note weaker filtered coagulase-preparation is sufficient to induce it to grow.
† Plate counts per 1.0 ml. serum.

TABLE 14

THE EFFECT OF pH-PRECIPITATED COMPONENTS UPON THE GROWTH OF SERUM-SUSCEPTIBLE STRAINS OF *MICROCOCCUS PYOGENES* IN NORMAL HUMAN SERUM

	Time (hrs.)	Control	pH 2.0 precipitate*	pH 8.0 precipitate
Strain no. 1.	0	3.0×10^2†	2.8×10^2	3.1×10^2
	24	5.2×10^3	6.0×10^7	8.0×10^2
Strain no. 2.	0	1.5×10^2	2.0×10^2	1.8×10^2
	24	2.5×10^2	8.5×10^7	3.2×10^2
Strain no. 3.	0	4.0×10^2	4.2×10^2	4.0×10^2
	24	3.5×10^2	7.0×10^6	6.0×10^2

* Coagulase activity: pH 2.0 precipitate—1:1024
pH 8.0 precipitate—1:8
† Plate counts per 1.0 ml. serum.

tered for coagulase activity by dissolving each at a concentration of 1.0 mg. per ml., diluting serially, and adding an equal volume of human plasma diluted 1:5. The results of these experiments are shown in TABLE 13, where it can be seen that the protective activity of the fractions and the coagulase activity are closely associated.

Precipitation of the crude saturated ammonium sulfate fraction from resistant-strain filtrate at various pH levels was also carried out. Two components separated out. One precipitated immediately at pH 2 and a second came down overnight in the cold at pH 8. The pH 2 component had strong coagulase activity, while the pH 8 component was lacking in this respect. Advantage was taken of this separation to test the neutralizing activity of these 2 components upon the antistaphylococcal substances of serum.

Three serum-susceptible strains of staphylococci were inoculated into human serum that had been treated with 1 mg. of the pH 2 precipitating material per ml. of serum. Serum was similarly treated with the pH 8 precipitating component and inoculated with the same strains. The growth of the organisms was followed over the usual 24-hour period. The results of these experiments are presented in TABLE 14 where it can be seen that the antistaphylococcal neutralizing capacity again is allied with the coagulase activity.

Discussion

The investigation presented here was begun with the thought of developing a more direct and, therefore, more reliable test for the potential pathogenicity of staphylococci. By studying the mechanisms whereby certain strains of these organisms were inhibited in their growth in normal human serum while others grew luxuriently, it became evident that coagulase, or a substance closely associated with coagulase, was in some way involved in this differential growth. A close correlation was noted between coagulase production and the ability to grow well in undiluted normal human serum. On the basis of the absorption experiments and of other evidence not considered here, it was concluded that the antistaphylococcal serum factor was of a generalized nature, possibly identi-

cal with the β-lysins of Pettersson (1924, 1926, 1928, 1936), and not specific in the immunological sense. It was postulated that coagulase functioned in some manner to protect those organisms that produced it against the antistaphylococcal activity of serum. To test this possibility, coagulase-negative or weakly coagulase-positive organisms that were susceptible to serum bacteriostasis were inoculated into serum to which had been added cell-free partially purified exogenous coagulase. Under such conditions these organisms were able to grow.

Further study of this protective capacity of coagulase strengthened our postulation. Other antigenic and metabolic constituents of the coagulase-positive, serum-resistant staphylococci, such as the Julianelle and Wieghard polysaccharides, α- and β-toxins, *etc.*, when tested for their ability to neutralize the antistaphylococcal activity of serum were incapable of so doing.

It must be made clear at this point that the coagulase preparations used in this study were not highly purified. In every case where we were successful in neutralizing the antistaphylococcal activity of human serum, however, the preparations from coagulase-positive serum-resistant organisms used to treat the serum had strong coagulase activity. Identically prepared fractions from coagulase-negative, serum-susceptible organisms were ineffective in neutralizing the serum factor.

These findings should be further substantiated by the use of highly purified coagulase preparations and, also, by inhibition of coagulase with anticoagulase antisera or other specific inhibitors. If a much more extensive screening of strains of staphylococci isolated from a variety of sources bears out the findings reported here, the *in vitro* tests to determine the *potential* pathogenicity of staphylococci might be further strengthened by determining their ability to grow in normal human serum. A certain level of coagulase production appears necessary for this growth. Weak coagulase producers are inhibited by serum and, although they are still considered pathogenic in the diagnostic laboratory, in fact they may be incapable of initiating an infection.

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THE FORMATION OF EXTRACELLULAR ENZYMES BY STAPHYLOCOCCI

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This work was undertaken primarily to further our understanding of the mechanisms underlying the formation of extracellular enzymes. Since, it still seems possible however, that some or all of the extracellular entities produced by staphylococci may be related to the ability of the organisms to harm the human host, it seems worth while to introduce, in this monograph, a few of the conclusions we have reached.

The types of approach that should be kept in mind when reading this summary of the work which is still in progress, are as follows: if the various active substances, such as coagulase, α-, β-, and γ-hemolysin, leukocidin, and hyaluronidase, that have been described as occurring in filtrates from overnight broth cultures of staphylococci, are wholly extracellular, then the organism that gains access to the body, denuded of its growth medium, cannot usually rely upon their help to initiate infection. If, on the other hand, these active substances also remain associated with the cell as a kind of capsule, they are likely to be as important for initiating, as for maintaining or spreading the organisms from the site of entry. The speed at which these various active substances are formed and accumulate may also be of importance to the invading parasite. A substance is clearly likely to be of most help to the attack on the host if it is formed faster than drainage from the area can remove it, or other defense mechanisms neutralize it. It is important to know the extent to which a strain can mutate to produce variants with different capacities to form the various aggressive agents. Also, attention has been paid to factors that can be recognized and estimated by these *in vitro* effects on body constituents, but we have no idea as yet whether these factors constitute the whole or only a small fraction of the extracellular armory of the staphylococci. No complete answers to these problems are contained here, but some guiding indications may be found.

Inhibition of the Formation of Extracellular Proteins

Any treatment of microorganisms that will selectively prevent their production of some biologically active proteins without affecting the formation of others is likely to prove a valuable tool in investigating both the pathological significance and the mechanism of formation of the proteins.

If staphylococci are coated with a layer of a highly negatively charged synthetic polymer, such as compound 531, described by Rogers and Spensley[1] in 1954, the formation of hyaluronidase and coagulase is almost completely inhibited.[2] The formation of α-lysin has also recently been shown to be inhibited. Growth, however, which involves the formation of many other proteins, proceeds at an unchanged rate. The inhibition of formation of the extracellular substances remains high, of course, only providing a comparatively small amount of growth takes place, otherwise the amount of inhibitor per cell be-

comes too small to be effective. All evidence points to the probability that these negatively-charged polymers, for which Spensley and I[3] have coined the term "macroanions," cause a true inhibition of the formation of the enzymes and other active extracellular substances and do not simply prevent their escape from the cells. Since considerable evidence exists that the polymer does not get into the cell, this implies that some stage in the formation of the extracellular substances takes place at or near the cell surface.

Other evidence suggests that extracellular substances such as enzymes, toxins, and hemolysins may be first extruded as a capsule that dissolves from its outer edge into the medium. This suggestion is in agreement with findings for both hyaluronidase[4] formation by staphylococci and extracellular penicillinase formation by *Bacillus cereus*.[5] The small amounts of these enzymes (the β-penicillinase only) that stay associated with the cells during growth and that have the same properties as those found in high potency freely in the culture supernatant, are neutralizable by antisera or are inhibited by the macroanions when examined while still *in situ*. They must therefore be somewhere near or at the cell surface. The small amounts of hyaluronidase that are associated with the cells in this manner, as a capsule, can be detected only during rapid growth of the culture and disappear as the culture ages.[5] After 18 hours' growth with aeration no enzyme can be detected at all on the cells despite very high concentrations in the solution. Thus rapidly growing cells are likely to be surrounded by a capsule of hyaluronidase and possibly other extracellular substances, whereas cells that have ceased to multiply rapidly are not.

Further examination of concentrated culture filtrates produced from media initially free of protein has been undertaken by the technique of paper electrophoresis. This technique has shown that at least 4 proteins are present in the filtrates from strain 524 SC 55.[6] The nature of these proteins is unknown, although presumably one is hyaluronidase. Examination of filtrates produced by growing 53D-treated cells showed that 3 of 4 of these spots had disappeared. Parallel examination of the total amount of extracellular trichloroacetic acid-precipitable protein present in filtrates from treated and untreated cells showed that this protein, persistently produced by the treated cells, accounted for almost 50 per cent of the protein formed by the control cells. Its nature is unknown but, since treatment of the cells by the macroanions is known to suppress formation of α-lysin, coagulase, and hyaluronidase, it is not likely to be 1 of these. Further examination of this material is being undertaken.

In view of the effects of synthetic macroanions on the formation and action of enzymes, hemolysins and other active proteins, it is of some interest to speculate on the effects of natural macroanions such as chondroitin sulphate (the major constituent of the ground substance of cartilage), hyaluronic acid, heparin, and the nucleic acids. Some experiments suggest that these substances too can inhibit the formation of active proteins by staphylococci and also stop their action when formed. Thus if an organism finds itself within a region in the body containing high concentrations of these substances it may well be unable to form, for example, α-hemolysin, unless it can form the other enzymes necessary to destroy the natural macroanions. Even if the hemolysin can be formed

it may not be able to act. Further possibilities of modification of the action of the enzymes and hemolysins are illustrated by the reversal of macroanionic inhibition by basic proteins such as protamine, and even by small diamines. Table 1 shows the inhibition of hyaluronidase by compound 53 and the reversal of this inhibition by protamine and a diamine. Thus the ability demonstrated *in vitro* to form an enzyme may only be part of the story since, in order to form active enzyme *in vivo*, other extracellular enzymes may be necessary to remove natural inhibitors such as macroanions, and the precise location of the organisms in the body may be as important as their enzyme-producing potentialities.

The Rate of Formation of Hyaluronidase

Despite the long history of the study of substances such as coagulase, hyaluronidase, and the hemolysins, precise studies on the rate of accumulation of the substances in the region of an increasing population of cells are remarkably few. In establishing an infection, however, this aspect is at least as important as the final potency achieved in an overnight culture of the organisms.

It was shown some time ago[5] that, in staphylococcal cultures growing in broth, the appearance of hyaluronidase first lags behind growth, but the enzyme is then formed about twice as fast as the mass of cells increases. Coagulase is formed immediately,[5-7] but at a rate somewhat slower than that at which growth occurs. The formation of any substance by cells at a rate faster than that at which they are growing leads to the interesting metabolic situation in which an increasing part of the cell machinery must be devoted to this process as the number of cells increases. Sooner or later, if growth is to be continued long enough, the rate must slacken. Otherwise gross metabolic imbalance will occur in the cells.

Table 1

The Reversal of the Macroanionic Inhibition of Bovine Testicular Hyaluronidase by Protamine and Pentadecamethylene-Diamine (P.D.D.).

53D (μg/ml.)*	Protamine (μg/ml.)*	P.D.D. (μg/ml.)*	TRU/ml.†	% Inhibition
0	0	0	1.05	—
50	0	0	0.37	64
50	5	0	0.37	64
50	50	0	0.85	19
50	100	0	1.12	0
0	50	0	1.25	Stimulated‡
0	100	0	1.37	"
0	0	0	0.78	—
50	0	0	0.42	45
50	0	30	0.58	26
50	0	50	0.68	13
50	0	150	0.75	5
0	0	100	0.79	0

* Final concentration in enzyme assay.
† Turbidity-reducing units of hyaluronidase in the procedure defined by Rogers and Spensley (1954).[1]
‡ Apparent stimulation due to combination of protamine with hyaluronate in the enzyme assay.

The effect of prolonged growth upon hyaluronidase formation by staphylococci was tested by growing them in a chemostat. The chemostat[8, 9, 10] is a device whereby microorganisms can be allowed to multiply at any rate up to the maximum characteristic of the medium for as long as is desired, during which time the opacity and composition of the culture can be maintained constant. When the potency of extracellular hyaluronidase is measured in such a chemostat culture, in which the cells are multiplying at a rate near to the maximum for the medium, it is found to increase about sixfold to eightfold during the first few hours and then become constant for as long as the culture is grown (FIGURE 1). Calculation has shown that 11 generations must elapse between the inoculation of the culture with cells from an overnight culture that has been grown in the ordinary way in a shaken flask of broth, and the point at which the rate of hyaluronidase formation slackens and becomes equal to that of growth. In other words, it takes the cells 11 generations, or approximately a thousandfold increase in mass, to overcome the effects of being left in an overnight culture. Further, it has been shown that if these cells, which have been grown in a chemostat until the rates of growth and enzyme formation are equal, are reinoculated into fresh broth, the rates remain equal. If, however, the chemostat culture is put into a flask and shaken in the ordinary way in the incubator for 16 to 18 hours and then inoculated into fresh broth, the whole phenomenon of a lag in hyaluronidase formation followed by a phase of more rapid formation is again reproduced. Thus some change takes place inside the cells during overnight incubation that it takes them 11 generations to forget. This phenomenon has not yet been fully explained, but 3 facts have been discovered that probably have some connection with it:

(1) Cells in an overnight culture accumulate some readily extractable substance that, when added to chemostat-grown cells, makes them mimic part of the behavior of the overnight culture cells.

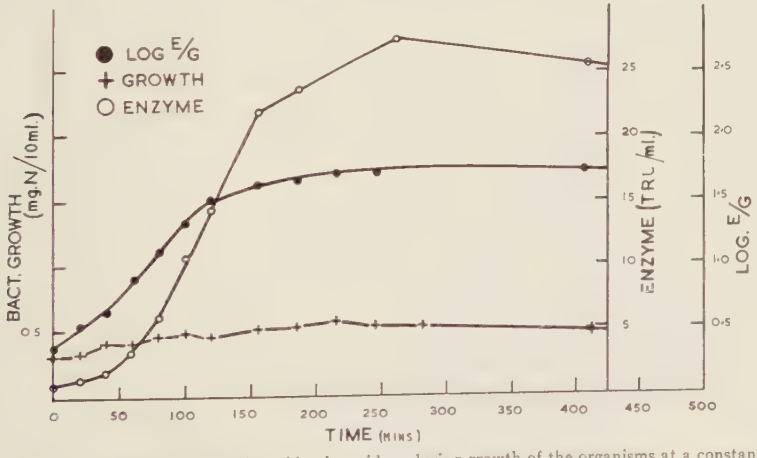

FIGURE 1. The course of production of hyaluronidase during growth of the organisms at a constant rate in a chemostat.
The line —+—+— represents the bacterial density in μg. bact. N/10 ml.
The line -○- ○— represents potency of extracellular hyaluronidase in TRU/ml
The line —●—● — represents ratio of hyaluronidase potency to bact. N/10 ml. This curve is plotted logarithmically.

(2) The substance α-aminobutyric acid accumulates in 18-hour cells and is not present in cells from the chemostat. This substance, in low concentration, somewhat inhibits hyaluronidase formation.

(3) Cells from an overnight culture contain a very low concentration of vitamin B_1 (thiamine). When placed in a fresh medium, the internal concentration rapidly increases to high levels. This occurs at very low culture densities and the external medium is left so deficient in vitamin B_1 that although reinoculation of overnight cells into a filtrate from such a young culture results in growth, very little hyaluronidase is formed. If such a culture filtrate is supplemented with 0.1 μg. ml. vitamin B_1, the normal amount of hyaluronidase is formed. Thus 2 different internal cell concentrations of vitamin B_1 are apparently required, a low one for growth and a much higher one for the formation of extracellular enzymes such as hyaluronidase.

Further work will no doubt show the relative importance of these 3 facts in explaining the phenomenon of an enzyme that is formed at a faster rate than the rest of the bacterial protein.

Variation in Cultures

It is common experience that the enzyme or toxic potency of strains changes during subculture, and it has been known for many years that cultures with different potencies can be produced from single colonies selected and grown from the parent culture. No conclusive experiments appeared until comparatively recently, however, that would allow us to decide the causes of this variation in cultures. The variation could be due either to uncontrolled variation of the medium used for testing single colonies selected from plate growths of the parent culture, or to the presence of a number of variants within the parent culture, each with its own enzyme-producing capacity. Present trends in bacteriology have emphasized the importance of the latter possibility, but precise proof is lacking, and studies of mutation of the type that would explain the formation of such clones with ability to produce different amounts of an enzyme or toxin are as yet comparatively rare,[11, 12, 13] even in species more amenable to genetic study. Accepting, however, the possibility of such mutants among bacteria, of a quantitative rather than a qualitative type, no evidence exists as to how many such variants or mutants are likely to establish themselves in a laboratory culture kept under the usual conditions. If, however, such a culture is plated and a large number of the resulting colonies is picked into broth, then a histogram can be drawn plotting the number of cultures, against the potency of extracellular hyaluronidase. This should give an indication of the number of variants present. Such a method is exactly analogous to the biological procedure for recognising the number of varieties in a mixed population of similar animals by measuring some recognized dimension. If the parent culture contains a small number of variants, a series of peaks in the histogram would be expected to appear, with a gaussian distribution of enzyme potency about them.

A culture of *Staphylococcus aureus* that had been isolated from a human infection by three successive single-colony selections and subsequently main-

tained for several months as a broth culture has been subjected to such examinations for hyaluronidase formation. Analysis of single-colony cultures from the original strain showed the presence of 4 distinct population types. The hyaluronidase potencies of the single-colony cultures that made up each population type had a gaussian distribution about the characteristic means of 20, 70, 150, and 300 turbidity reducing units (TRU) ml. for the 4 types respectively. Two pairs of cocci were isolated by micromanipulation from the population, having the mean of 300 TRU ml. These cells were chosen from individual cultures giving potencies toward the ends of the distribution curve shown in FIGURE 2. The same type of population (that is, the type yielding single-colony cultures with a mean of 300 TRU ml.) was found to develop from both of these pairs of cells. A culture from the population has been maintained dried (Stamp[13]) for some 4 years with intermittent growth and redrying. When grown under the same conditions as those used for the above selection, a potency

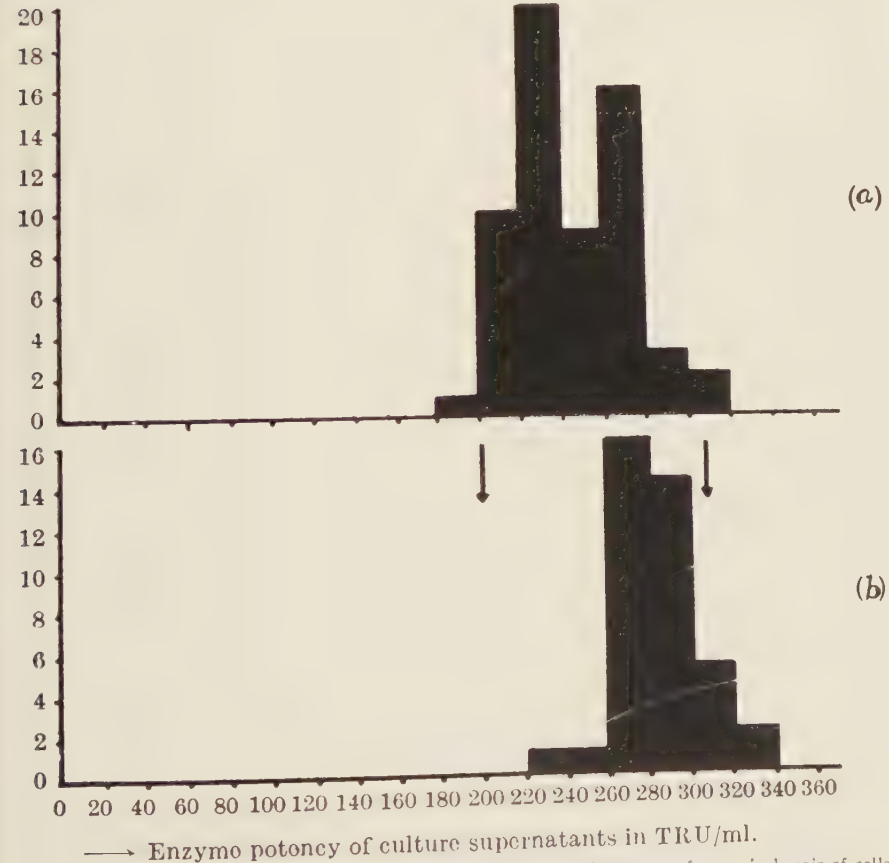

FIGURE 2. A histogram of the potency of cultures obtained from a culture grown from a single pair of cells (b), together with the populations (a) from which these were selected. ↓ indicates the potency of the cultures from which the 2 pairs of cells were isolated. From the *Journal of Pathology and Bacteriology* **66**: 545.)

of 300 ± 30 TRU ml. is still obtained. The variants recognized in the original culture were all identical in characteristics other than hyaluronidase formation, including phage susceptibility.

It accordingly seems likely that many cultures that have been allowed to grow under conditions such that the enzyme, toxin, or hemolysin under study provides the organism with no selective advantage or disadvantage, consist of a small number of variants of widely differing ability. In the event of the organism being placed under conditions where the active substance is of advantage, the variant that either produces most of it or produces it at the most advantageous rate will be selected.

All the above observations tend to stress the importance of a more intense analytical approach to the problem of the relation of enzymes or toxins to the pathogenic process. The correlation or the absence of correlation between the potency of any substance X found in the supernatant from an overnight broth culture of the organisms and the pathogenic abilities of the strain may well be quite fortuitous. This, of course, leaves out of account the further probability that substances may be produced *in vivo* that never appear in broth cultures at all.

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THE CHEMISTRY OF STAPHYLOCOCCAL ENTEROTOXIN*

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Staphylococcal enterotoxin is recognized as the most common cause of food poisoning in the United States. More recently this toxin has been implicated in gastrointestinal upsets in a few individuals who have received large doses of antibiotics.[1]

A number of investigators have attempted to determine the chemical nature of enterotoxin, but for the most part these studies have been inconclusive. One of the difficulties encountered has been the lack of an adequate assay procedure. Intravenous and intraperitoneal injections into cats and kittens have been carried out by many investigators. Feeding of young monkeys (*Macaca mulatta*) has been used to a lesser extent because of the cost and upkeep of these animals. My colleagues and I have used the monkey in our work because we believe it to be the most reliable test animal for enterotoxin.

Our assays for the presence of the toxin are made by feeding the samples in solution (usually 50 ml.) to young monkeys by stomach tube. The animals are observed for 5 hours after feeding, and vomiting is accepted as a positive reaction for enterotoxin. This method has been reviewed in detail.[2]

One of the purposes of our investigations was to develop a quantitative assay procedure for enterotoxin. To accomplish this, several approaches have been followed. One was a study of the chemistry of the toxin, and this report outlines the advances in that field. In this connection, Levi, Matheson, and Thatcher[3] recently reported that enterotoxin could be detected by infrared spectrophotometry. The intensity of the absorption in the 1100 to 1000 cm.$^{-1}$ region was higher in toxic than in nontoxic preparations. Further evidence is needed to prove that the absorption was due to enterotoxin.

A second approach has been through mode-of-action studies. Sugiyama[12, 13] has conducted numerous investigations in this area.

A third approach has been through immunochemistry. Early studies indicated that a sensitive assay procedure might be developed by using the toxin-antibody reaction. The value of such a method may be limited somewhat by the discovery that enterotoxin produced by 1 strain of *Staphylococcus* may be antigenically different from that produced by another strain.[4, 5] Although an assay procedure utilizing immunochemistry has not been developed as yet, the technique of Oudin,[6, 7] employing antigen-antibody reactions in agar, has been adapted for use in following the purification of the toxin.[4, 5] In brief, this technique involves the layering of an agar column containing antiserum with an antigen solution. Under favorable conditions a band of precipitate forms in the agar for each antigen-antibody system present. By the process of elimination in 1 preparation, we tentatively identified 1 specific band as the enterotoxin-antienterotoxin precipitate. Definite proof awaits the completion of the immunization studies in which a serum with only antibodies to enterotoxin is obtained.

* This work is supported by organizations concerned with the food industry.

Our studies indicated that enterotoxin was produced in very small quantities in culture media. In order to accumulate sufficient enterotoxin for the completion of the purification studies, pilot-plant production was undertaken. Several thousand gallons of broth medium were used for growing cultures to prepare a stock sample of partially purified enterotoxin. The methods of production of enterotoxin have been published elsewhere.[9]

All methods commonly employed in the fractionation of proteins were applicable to the purification of enterotoxin.[10, 11] Several of these procedures were combined into a series of steps to concentrate the toxin produced in the pilot plant. This series of steps was: (1) precipitation at pH 3.5 with H_3PO_4; (2) filtration through Hyflo Super-Cel; (3) extraction of the precipitate with phosphate buffer; (4) adsorption of the enterotoxin in 0.035 M, pH 6.2 phosphate buffer by alumina followed by elution with 0.2 M disodium phosphate; (5) precipitation of the enterotoxin in the eluate from 40 per cent ethanol at pH 5.5 and $-7°$ C.; (6) dialysis; and (7) lyophilization. The yield of partially purified enterotoxin was approximately 5 mg. of material per liter of bacterial filtrate.

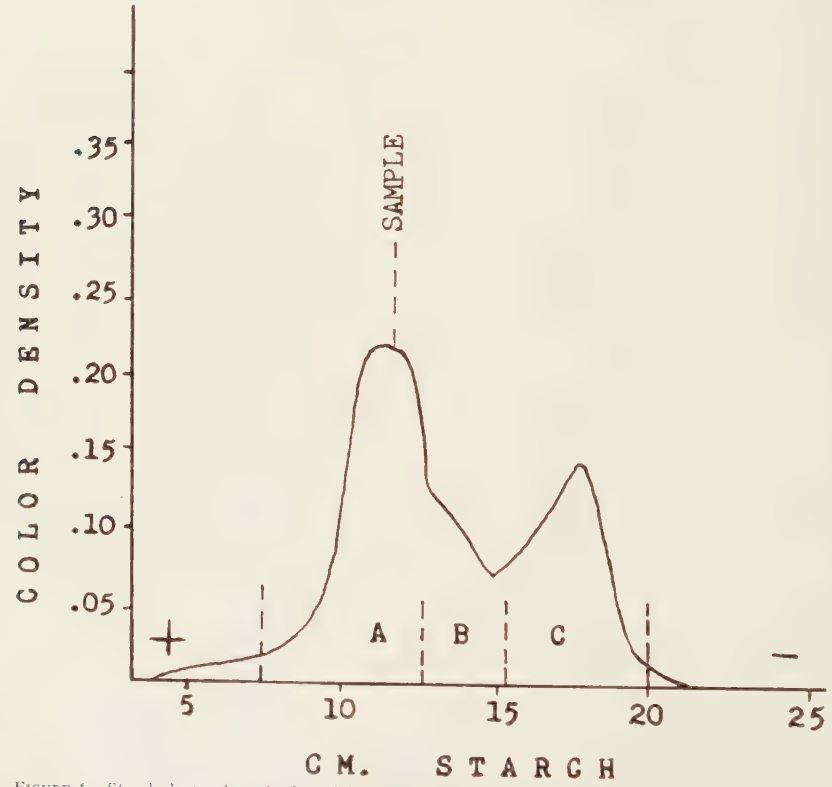

FIGURE 1. Starch electrophoresis of partially purified enterotoxin. Starch bed size: 27 × 27 × 2 cm. Buffer: 0.05 M, pH 6.0 sodium phosphate. Development conditions, 60 V, 62 ma., 24 hours. Color reagent: modified Folin.

The above preparation was a mixture of water-soluble, heat-coagulable proteins of an estimated molecular weight of 15 to 25,000. The gel-diffusion technique indicated the presence of at least 6 antigens. Electrophoresis separated the material into 2 fractions, 1 with an isoelectric point near pH 5.0 and the other with an isoelectric point near pH 8.5. The latter fraction contained most of the enterotoxin. Treatment with trypsin did not destroy the enterotoxin, but did slowly digest the other antigenic substances present. The preparation contained 15 per cent nitrogen, 18 per cent lysine, and an estimated 10 to 20 per cent enterotoxin. As little as 50 gamma of this material caused emesis in monkeys. The presence of such biologically active substances as fibrinolysin, coagulase, apyrase, and α-lysin has been demonstrated.[12, 13] This stock sample was used in subsequent experiments.

Further purification of enterotoxin was accomplished primarily with 2 procedures, namely starch electrophoresis and ion-exchange chromatography. Electrophoresis with paper as the supporting medium has been used extensively for the separation of proteins. Our attempts to fractionate enterotoxin with this method were unsuccessful. More recently, Thatcher, Matheson, and Simon[14] have also attempted to use this method to fractionate enterotoxin, but their recovery of active toxin was unsatisfactory.

Electrophoresis with starch as the supporting medium has become a valuable tool for the fractionation of protein mixtures in gram quantities. Partially purified enterotoxin can be separated into 2 fractions by this method (FIGURE 1). A wide range of conditions has been used with little apparent change in the final results. The properties of the fractions are listed in TABLE 1. Enterotoxin was present as the major component in the fraction that moved toward the cathode. The usefulness of this method for the purification of the toxin has been limited because of the low recovery of active material. There was some indication that the toxin was being separated from a protective substance(s) during the electrophoresis.

Chromatography with the cation-exchange resin, Amberlite XE-64, (IRC-50), has been used to isolate a number of basic proteins. Partially puri-

TABLE 1

PROPERTIES OF FRACTIONS FROM STARCH ELECTROPHORESIS AND AMBERLITE XE-64 CHROMATOGRAPHY

	Starch electrophoresis			Amberlite XE-64 chromatography				
	Fraction							
	A	B	C	A	B	C	D	E
Per cent of nitrogen	35.0	6.0	20.0	45.0	7.5	4.3	4.0	1.3
No. antigens	4	6	5	5	3	3	4	2
Isoelectric pt.	5.0		8.5					
Enterotoxin	0	+	+++	+++	+++	++	+	0
Apyrase	0	+	+++	+++	++	+	+++	0
Coagulase	+++	+	0	+++	0	0	0	0
Fibrinolysin	+++	+	0	+++	+	+	+	+

fied enterotoxin has been separated into 5 fractions by this method (FIGURE 2). The properties of these fractions are listed in TABLE 1. Fractions B and C contained enterotoxin as the major component, while A and D contained the toxin in lesser proportions. Rechromatographing of A, B, and C indicated that these fractions were distinctly different. Use of the gel-diffusion comparator cell showed, however, that the enterotoxin in each of these fractions did react with the same antibody. Usually a considerable loss of activity occurred during the chromatographing. The loss in activity in both the electrophoresis and chromatographic procedures has delayed the isolation of pure toxin until the nature of the inactivation can be determined and counteractive measures can be devised.

Sufficient information has accumulated to indicate that enterotoxin has the following properties: (1) it is a water soluble protein of a molecular weight of 15-25,000; (2) it has an isoelectric point of near pH 8.5; (3) it is antigenic; (4)

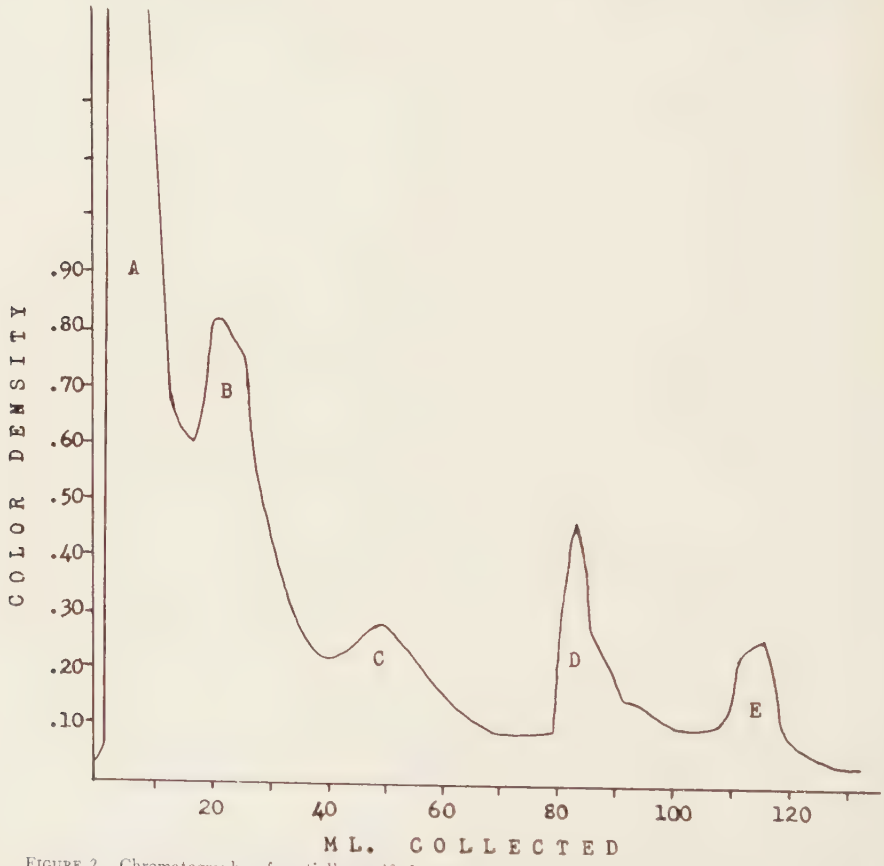

FIGURE 2. Chromatography of partially purified enterotoxin on the ion-exchange resin, Amberlite XE-64. Column size: 0.9 × 7.5 cm. Buffer used to wash resin and develop column until 80 ml. were collected: 0.05 M, pH 6.8 sodium phosphate. Buffer used to finish development of column: 0.2 M, pH 7.4 sodium phosphate. Rate of percolation: approximately 25 ml. per hour Color reagent: modified Folin.

it contains a high percentage of lysine; and (5) it is trypsin resistant. Although none of these properties lend themselves to the development of an assay procedure, some distinguishing characteristics of the enterotoxin molecule may be found by the chemical studies now in progress.

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Part III. Immunity, Epidemiology, and Antimicrobial Resistance

THE ROLE OF COAGULASE IN STAPHYLOCOCCAL INFECTIONS*

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The characteristic and intriguing feature of staphylococcal infections is the focal abscess. It has been suggested that the extracellular product, coagulase, is the factor produced by the *Staphylococcus* that is responsible for the localization of the bacteria within the inflammatory process. The laying down of a fibrin barrier that protects the *Staphylococcus* from the cellular defense mechanisms and allows multiplication of the bacteria in the inflamed tissues is an attractive hypothesis. Although most strains of staphylococci isolated from the focal abscess produce coagulase, it was not until 1947 that evidence was obtained that implicated the coagulase mechanism in the evolution of the abscess. Smith, Hale, and Smith[1] were able to demonstrate that phagocytosis of various strains of staphylococci was inhibited by the coagulase system. Of even greater importance were their observations on artificial infections in the subcutaneous tissues of guinea pigs. The infections were produced by a strain of *Staphylococcus* that was coagulase-positive when tested in human plasma, but was coagulase-negative against guinea pig plasma. Following subcutaneous inoculation of this organism an inflammatory response was observed that subsided within a few days without the development of abscesses. If human plasma was injected, however, and then a broth culture of *Staphylococcus* was introduced into the same site, multiple abscesses developed.

Because these studies suggested that a functioning coagulase mechanism was important in the evolution of the staphylococcal abscess, a series of investigations in animals and man was undertaken in our laboratories. The effect of anticoagulase and reacting factor on the development of staphylococcal abscesses was studied, since the coagulase reaction with fibrinogen is altered by the concentration of these 2 substances in the system. The monkey was selected as the ideal experimental animal for the study of the role of anticoagulase. The injection of coagulase-positive staphylococci into the subcutaneous tissues of monkeys usually produces an abscess and, following recovery, type-specific anticoagulase can be demonstrated in the serum.[2]

An example of the clinical and immunological response to staphylococcal infection is shown in FIGURE 1. Broth cultures of staphylococci producing coagulase of types I and II were injected subcutaneously.[2] At the time of inoculation the monkey showed antibodies to coagulase I, but not to coagulase II. The inflammatory response to the 2 infections was different. A large draining boil was produced by type II organisms in contrast to the small ab-

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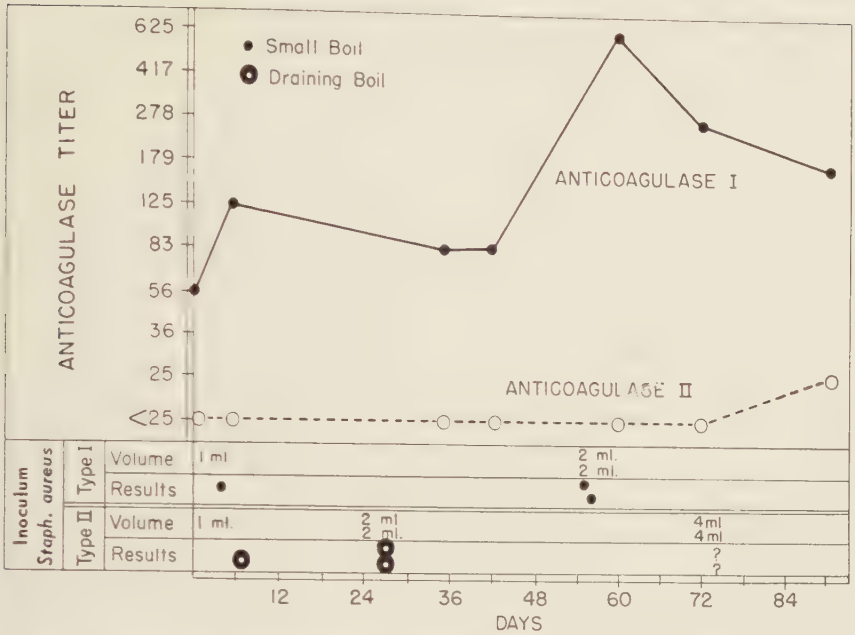

FIGURE 1. Serological and clinical response of a monkey to injection of cultures of *Staphylococcus aureus*.

scess that developed at the site of injection of type I staphylococci. Subsequently, the injection of increased numbers of type II staphylococci produced large draining abscess but, again, the response to type I organisms was minimal. Type-specific anticoagulase developed to each organism. In other experiments monkeys were immunized with cell-free coagulase prior to infection but, in spite of the presence of high titers of antibody, it was not possble to prevent abscesses in every instance. These studies were discontinued because of the varying clinical response to infection in normal animals and the lack of complete protection against abscess formation in animals showing high titers of anticoagulase.

In the preliminary studies on the role of reacting factor in staphylococcal infections, the chicken, duck, and goose were selected as experimental animals. The serum from these birds contains little or no reacting factor, and no abscess develops following the injection of coagulase-positive strains. The injection of reacting factor in the form of human serum failed to alter the inflammatory response to infection. In these studies, however, the serum was not injected into the site of infection. This model was abandoned when it was observed that the growth of coagulase-positive staphylococci *in vitro* was inhibited by chicken, duck, and goose blood. This inhibitory effect could be demonstrated in systems that excluded participation of cellular elements. Thus, the failure to produce an abscess or, indeed, even severe inflammatory reactions in these birds may be due to the natural inhibitory properties of the serum and not to some defect in the coagulase-reacting-factor mechanism.

An indirect approach to the study of the role of the coagulase-reacting-factor mechanism in abscess formation was afforded by the vagaries of these infections in man. It is our impression that the staphylococci usually do not cause an abscess in the infant. Rather, the inflammatory reaction tends to be diffuse and might be compared to the infection observed by Smith, Hale, and Smith[1] in guinea pigs. In the young child, abscesses are not common, but it is in this age group that bacteremia and infection of the bone develops. In the adult, boils and multiple abscesses are common, but osteomyelitis and bacteremia are rare. The explanation for these different patterns of illness in the child and in the adult is not known but, presumably, in some manner age may alter the ability of the individual to localize the infectious process.

If the coagulase-reacting-factor reaction is responsible for the development of abscesses in the adult, it is possible that some defect is present in the infant or in the child who develops osteomyelitis so that a fibrin barrier is not deposited around the bacteria. Theoretically, the absence or decrease in the amount of reacting factor or the presence of specific or nonspecific inhibitors might explain the lack of abscess formation in the child. In the present study a method was devised to measure the reacting factor content of blood. The results obtained in a survey of normal individuals are presented because they show certain variations that may be related to the type of illness observed in the young child as compared to the adult.

Methods

Coagulase. A strain (No. 58) of *Staphylococcus aureus*, which produces a coagulase of type III, was selected for these studies.[2] The coagulase produced by this strain is potent, and few human sera exhibit specific inhibitors to it. Approximately 0.2 ml. of an overnight tryptose broth culture of strain 58 was inoculated into 500 ml. of tryptose broth containing 5 per cent bovine albumin.* Albumin was used in the medium because it enables coagulase to pass a Seitz filter and, unlike plasma, it contains little or no reacting factor. After 3 days incubation at 37° C. the staphylococcal cells were sedimented by centrifugation, and the supernatant was passed through a Seitz filter. The first 50 ml. were discarded, and the remaining amount was stored in sealed ampules or flasks at either −4° C. or 4° C.

Buffered saline. A solution of 0.01 M phosphate in 0.85 per cent sodium chloride at a pH of 7.4 was employed.

Reacting factor. A standard preparation of reacting factor in the form of serum from 1 individual was used for the standardization of coagulase. Previous studies have indicated that this reacting factor was stable when stored at 4° C. for a period of at least 1 year.[3]

Fibrinogen. A solution of bovine fibrinogen* (fraction I) containing 100 mg. in 63 ml. of buffered saline was placed in a water bath at 37° C. for 30 minutes. One ml. of buffered saline containing 0.1 mg. of heparin was then added to the fibrinogen solution to prevent clotting caused by an occasional serum specimen. This preparation of fibrinogen was used within a period of 4 hours.

* Armour and Company, Chicago, Ill.

TABLE 1
REACTING-FACTOR CONCENTRATION OF 4 CONTROL SERA AS DETERMINED ON DIFFERENT DAYS

Date	Units of reacting factor			
	Serum A	Serum B	Serum C	Serum D
11/17/48	3200	1200	1600	3200
11/18/48	3200	1200	1600	3200
11/19/48	3200	800	1600	2400
11/20/48	3200	1200	1600	3200
12/ 2/48	3200	800	1600	2400
12/ 6/48	3200	800	1600	3200
3/19/49	2400	800	1600	—

Standardization of coagulase. A preliminary test to determine the activity of the cell-free coagulase was performed by making serial twofold dilutions in 1.0 ml. of tryptose broth. To each tube was added 1.0 ml. of the fibrinogen solution containing a 1:3500 dilution of the standard reacting factor. This concentration of reacting factor was arbitrarily designated as 1 unit. The tubes were incubated for 3 hours at 37° C. in a water bath. The highest dilution of coagulase that produced a visible shred was then used for the final titration. In a duplicate series of 8 tubes, 0.1 ml. decrements of coagulase were added, and the volume in each tube was adjusted to 1.0 ml. with tryptose broth. One ml. of the fibrinogen solution containing 1 unit of reacting factor was added. After incubation for 3 hours at 37° C. in the water bath, the tubes were read with the aid of a hand lens. A unit of coagulase was defined as the smallest amount that produced visible shreds of fibrin under these standard conditions.

Measurement of reacting factor. The reacting factor of an unknown serum or plasma was measured by its ability to react with 1 unit of coagulase under stand-

TABLE 2
REACTING-FACTOR CONTENT OF SERA FROM NORMAL SUBJECTS ACCORDING TO AGE

Units reacting factor	Age of normal subjects		
	< 2 years	2–15 years	>15 years
100	7	2	
200	2	2	
300	4	0	
400	4	4	1
600	2	1	
800	3	4	1
1200	2	6	2
1600	2	27	9
2400		10	13
3200	1	20	24
4800		5	8
6400		8	4
Total	27	89	62

Table 3
Reacting-Factor Content of Several Serum Specimens Obtained from the Same Individual

Subject	Age	Date	Reacting factor, units/ml.
	mos		
F15	15	7/22/47	300
	24	7/26/48	600
	29	9/13/48	1600
	35	3/21/49	1600
	yrs.		
F31	32	7/25/47	3200
		4/28/49	3200
F161	43	1/10/48	6400
		5/14/49	6400
J.B.	42	7/ 1/48	2400
		7/14/48	2400
		7/27/48	3200

ard conditions. A 1:100 dilution of the unknown serum or plasma was prepared by adding 0.1 ml. of the specimen to 10 ml. of the standard fibrinogen solution containing heparin. Serial twofold dilutions in 1 ml. of standard fibrinogen solution were then made in 2 series of 7 tubes. The initial dilution in the first tube in 1 series was 1:100, in the second series 1:300. One ml. of the coagulase preparation in tryptose broth containing 1 unit was then added to

Table 4
Reacting-Factor Content of Serum from Patients with Primary Atypical Pneumonia

Patient	Day of disease	Strep. MG agg. titer*	Cold agg. titer*	Reacting factor, units
2776	6			100
	13	640	256	1200
	20	320	128	1600
	38	320	0	3200
3412	2	0	16	800
	7	0	32	1200
	14	0	128	1600
	21	0	0	3200
2462	5	0	0	400
	12	0	128	600
	19	0	256	2400
2302	6	0	0	800
	13	20	128	400
	20	80	2048	2400
	34	20	64	1600

* Agglutinins for *Streptococcus* MG (a strain originally isolated at the Rockefeller Institute of Medical Research, New York, N. Y.) and cold hemagglutinins.

each tube with an automatic pipette. After incubation for 3 hours at 37° C. in the water bath the tubes were read with the aid of a hand lens. The highest dilution of serum or plasma that resulted in the formation of visible shreds was defined as 1 unit of reacting factor.

One or more control sera of known reacting-factor concentration were included in each titration. The concentration of reacting factor in 4 such controls is recorded in TABLE 1. It is apparent that the measurement of reacting factor on different days was accurate, and that in no instance was a twofold variation in reacting-factor content observed.

Results

The sera collected from normal adults usually showed high concentrations of coagulase reacting factor (TABLE 2). In 93 per cent of the specimens examined the sera contained at least 1600 units. In the child the concentration of reacting factor was less. In this age group, 78 per cent showed concentrations of at least 1600 units, while several individuals exhibited little reacting factor. The infant under 2 years of age usually showed small amounts of reacting factor. In approximately half of the specimens obtained from infants there were less than 400 units, and only 3 individuals exhibited concentrations of 1600 units or greater.

That the reacting-factor content increased with age is also shown in TABLE 3. In the infant the reacting factor increased from 300 units to 1600 units over a period of 20 months. In adults the content of reacting factor in the serum

TABLE 5

REACTING-FACTOR CONCENTRATION OF SERUM FROM PATIENTS WITH INFECTIOUS HEPATITIS AND LEPTOSPIROSIS

Patient	Day of disease	Reacting factor, unit
TM*	12	300
	15	800
	23	3200
	31	1600
	36	4800
DP*	9	400
	17	100
	31	600
MK*	8	<100
	11	<100
	19	<100
	28	2400
	34	3200
JL†	14	100
	23	<100
	30	<100
	51	3200
	64	3200

* Infectious hepatitis.
† Leptospirosis (sera obtained from Paul Beeson).

appeared to be relatively constant, being altered only during certain disease states. For example, TABLE 4 shows the changes in reacting factor observed in patients with primary atypical pneumonia. Similar depressions in reacting factor were regularly observed in patients with infectious hepatitis and Weil's disease (TABLE 5).

Discussion

Inhibitors to coagulase and to reacting factor may interfere with the measurement of reacting factor. The coagulase inhibitors, anticoagulases, are type-specific[2] and therefore a coagulase type against which few sera exhibit antibodies should be selected for the determination of reacting factor in specimens obtained from man. The presence of specific anticoagulase in the serum specimen may be detected by noting the absence or paucity of clot formation in the low dilutions in the reacting-factor test. In addition, the anticoagulase titer of the serum may be measured after removal of the reacting factor by a method described previously.[3]

Tager and Hales[4] have indicated that in certain specimens of plasma the coagulase reacting factor may be bound or inhibited by other substances in the tissues. They showed that acid precipitation apparently released the reacting substance in the few samples of chicken and guinea pig plasma tested. The role of such inhibitors in the sera was not determined in the present study.

In this investigation the reacting-factor content of sera from adults was usually above 1600 units. In children it was not unusual to observe low concentrations and, in the infant, small amounts were the rule. The concentration of reacting factor increased with age and was relatively constant in the individual adult. Occasionally during the course of certain infectious processes a temporary suppression of reacting factor was observed. Since these studies showed some relationship between the concentration of reacting factor and the nature of the staphylococcal infection observed among individuals of various age groups, it seemed possible that the coagulase-reacting-factor system may play a role in the localization of these infections in man. In this regard, it would be important to ascertain the concentration of reacting factor and specific anticoagulase in children exhibiting various forms of staphylococcal infections. Until this is accomplished it is difficult to define the exact role of coagulase in staphylococcal infections in man.

Since these studies were completed, Tager and Lodge[5] and Duthie and Lorenz[6] have shown that the reacting factor content of serum is lower than that observed in plasma. Reacting factor is consumed during the clotting process so that the amount of reacting factor found in serum may reflect alterations in prothrombin conversion. Thus, the small quantities of reacting factor observed in sera from infants and children and in sera from patients with atypical pneumonia, infectious hepatitis, and leptospirosis cannot be interpreted as absolute deficiencies. Further studies of the reacting factor in plasma are required. It appears possible, however, that such investigations might prove profitable since Miale[7] has recorded that the plasma obtained from patients with infectious hepatitis contains only small amounts of reacting factor.

Acknowledgment

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EPIDEMIOLOGICAL IMPLICATIONS OF STAPHYLOCOCCAL PHAGE TYPING

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Staphylococci are constantly present in man's environment and, under normal conditions, are carried in the nose or throat of well over one half of all individuals. This fact suggests the apparent ease with which staphylococci can be disseminated and, of more importance, that attempts to determine the source or to trace the spread of staphylococcal infections sometimes can be confused by the presence of strains that may have no epidemiological relation to the problem under investigation. In searching for the source of a staphylococcal infection, therefore, it is not enough simply to demonstrate that staphylococci are present, for example, in the nasopharynx or on the skin of either the patient or his contacts, or that staphylococci are present in the environment. It would be much more significant to show that strains identical with the specific strain responsible for the infection are present in cultures from these general sources with at least a fair degree of consistency.

Epidemiological investigations of staphylococcal infections have been hampered seriously in the past by the lack of a suitable method of demonstrating the identity or nonidentity of individual cultures. While certain biological characteristics of the staphylococci are useful for identification of the species, they are not particularly helpful when one is faced with the problem of deciding which of several cultures from related sources are identical and which are different.

The problem of differentiation of cultures of staphylococci has been aided and clarified by bacteriophage typing. The particular value of phage typing lies in its ability to demonstrate the identity or nonidentity of sets of cultures. This can be done with a reasonable degree of accuracy. The significance of the results obtained by phage typing is enhanced by the fact that both the specific action of the phages and the patterns of susceptibility of cultures to the phages are relatively stable properties.

Space does not permit a detailed review of the many practical applications of staphylococcal phage typing that have been reported in the literature. Instead, it is the purpose of this article to discuss briefly the incidence and distribution of phage types of staphylococci. While much remains to be learned through the further application of this relatively new procedure, the information that has been gathered has already thrown light on some problems relating to the epidemiology of staphylococcal infections. These studies assume an added significance when the phage typing of cultures of staphylococci is combined with the determination of their sensitivity or resistance to the antibiotics.

Reference should be made to the "basic" phages that are recommended at present for routine typing by the Subcommittee on Bacteriophage Typing of Staphylococci, of the International Committee on Bacteriological Nomenclature, London, England. The basic series is composed of 19 phages that are

divided into 4 Groups, as follows: Group I, phages 29, 52, 52A, and 79; Group II, phages 3A, 3B, 3C, and 55; Group III, phages 6, 7, 42E, 47, 53, 54, 70, 73, 75, and 77; and Group IV, phage 42D. This grouping is now recognized by most workers in the field. Essentially it follows the scheme that has been employed by Williams and his associates[1] in the Staphylococcal Reference Laboratory of the Public Health Laboratory Service in London, England. The Committee suggests that, in addition to the basic series, other phages may be employed when the investigator feels that they will provide useful additional information. Accordingly, by way of illustration, the author currently is using 5 additional phages: 39 and 523 in Group II, 42B and VA4 in Group III, and 44A, the last being presently assigned to a miscellaneous group, unrelated to the 4 broad groups described above. These 5 phages also were used in work previously reported by Blair and Carr.[2]

The techniques of phage typing of staphylococci have been described in detail by Williams and Rippon,[3] Blair and Carr,[2] and Jackson, Dowling, and Lepper.[4] Cultures are assigned to a broad phage group on the basis of their lysis by 1 or more of the phages that identify that group. While some cultures are lysed specifically by a single phage, it often is found that lysis is produced by 2 or more phages within a broad group to give so-called "patterns of lysis." Identical strains of staphylococci show identical or closely similar patterns, while differences between cultures are shown by differences in their patterns of lysis. Phage typing thus indicates the broad group to which a culture belongs, while fine distinctions within the groups are based upon the patterns observed. It is in the ability to differentiate individual cultures within a broad group that phage typing has a significant epidemiological application.

There is a considerable degree of group specificity. Cultures that are lysed by the phages of Group II, for example, are not susceptible to lysis by the phages that identify the other broad groups, and so on. Occasional strains are encountered, however, that are lysed by phages of Groups I and III. These seem to occupy an intermediate position that has not yet been exactly defined.

It must be pointed out that susceptibility to the typing phages is a characteristic of the coagulase-positive, that is, potentially pathogenic, staphylococci. Coagulase-negative typable strains are extremely rare, if, indeed, they exist at all. A certain proportion of coagulase-positive cultures are not susceptible to the typing phages. This proportion may be as high as 30 per cent in a large series of antibiotic-sensitive cultures, but often is as low as 10 per cent or less when antibiotic-resistant strains are subjected to phage typing. The great majority of typable cultures belong to Groups I, II, or III. Strains of Group IV appear to be generally less common and, as mentioned above, occasional cultures are found to be lysed by phages of both Groups I and III.

The incidence of staphylococci of the several phage groups shows a rather distinct difference when cultures that are isolated in hospitals are compared with those that are obtained from the general population. Staphylococci that are present in cultures taken on admission to the hospital, either from the nose and throat of the patients or from primary infections acquired outside of the hospital, may be regarded as indicative of the strains that exist among the

general population. Jackson, Dowling, and Lepper[5] found a close similarity between the staphylococci in 153 cultures of the noses or throats of patients, taken on admission, and those of their household contacts. Staphylococci isolated from both the patients and their family contacts were distributed through all the broad phage groups and were represented in each group in comparable proportions. The patterns of lysis exhibited by these cultures were varied. Five patterns were found in Group I, although 90 per cent of the cultures were lysed specifically by phage 52; 6 patterns occurred in Group II, 59 per cent of the cultures being lysed by phage 3C; and as many as 49 patterns were observed in Group III, 2 patterns being especially characteristic of cultures from the throat.

Williams, Rippon, and Dowsett,[4] in England, found that staphylococci in cultures of the noses of healthy individuals were most commonly of Group I. Although Rountree,[6] in Australia, found a fairly large proportion of cultures from this source in Group I, a higher proportion belonged to Group III, an observation that was also made by Jackson and his associates.[5] The difference between the English and the Australian and American observations could well be due to differences in the populations examined. The results of Jackson and his associates substantially confirm those reported by Williams and his associates, however, for Group I strains were found to occur in significant numbers in the respiratory tract more often than would be expected from random distribution.

In a study of all consecutive cultures of coagulase-positive staphylococci isolated in the diagnostic laboratory during the past year, the author has found a similar distribution of staphylococci in admission cultures through all phage groups, as well as a variety of phage patterns within each group. In this study, approximately 63 per cent of the admission cultures were sensitive to the antibiotics. The variety and distribution of phage types found among the general population does not appear to have changed materially during the past 10 or 15 years. A variety of phage patterns was found by Knight and Holzer[7] in a series of 55 cultures of *Staphylococcus aureus* that had been collected by the late Ward J. MacNeal between 1932 and 1948. Of these cultures, 88 per cent were sensitive to penicillin. A comparable distribution and variety of phage patterns was exhibited by 66 cultures of coagulase-positive staphylococci that were isolated by the author during the "preantibiotic" days and had been preserved for study. The varied phage patterns that occur in admission cultures, especially among the antibiotic-sensitive strains, suggest that a rather heterogeneous collection of staphylococci exists in the general population.

The picture is somewhat different when phage typing is applied to coagulase-positive *Staphylococcus aureus* isolated from patients who have been in the hospital for a week or more, or from members of the hospital personnel. In the first place, the majority of strains isolated from these sources are resistant to 1 or more of the antibiotics. The high incidence of antibiotic resistant staphylococci now being encountered in hospitals is common experience. This finding has been documented in numerous reports and needs no detailed discussion here.[6-13]

Of considerable significance is the fact that the great majority of antibiotic-resistant staphylococci isolated in hospitals belong to phage Group III. This has been an almost universal observation wherever phage typing has been applied to studies of the incidence of staphylococci.[2, 5, 6, 7, 9] Next in order of frequency are antibiotic-resistant staphylococci of Group I.[6, 7] Under special conditions, resistant strains of this group have been found to predominate in cultures isolated in a hospital ward or nursery.[14, 16] Antibiotic-resistant strains of Group II are rare. The author has not encountered a Group II antibiotic-resistant strain in several hundred cultures isolated in the diagnostic laboratory of the Hospital for Joint Diseases, New York, N. Y., but he did have the opportunity to examine 1 such strain that was carried by 5 individuals in a hospital in another city. Group II strains of staphylococci that were resistant to streptomycin were present in 1.5 per cent of 516 cultures isolated in a hospital by Knight and Holzer.[7] Among 1192 penicillin-resistant cultures from varied pathological conditions that were examined by Rountree,[6] the incidence ranged from 0 to 3.4 per cent, and was only 0.5 per cent in 273 strains isolated from the noses of members of the hospital staff. Barber and her associates[17] reported an incidence of 3.3 per cent of Group II strains in a series of 240 penicillin-resistant cultures.

When strains of coagulase-positive staphylococci carried by the patients and the hospital personnel are compared, the distribution and incidence of the phage groups often is found to be parallel. Jackson and his co-workers[5] found an incidence of 16.4 per cent of Group I strains in the noses and throats of patients, and of 9.8 per cent in the personnel. In Group II the incidence was 3.5 and 2.8 per cent, respectively, and in Group III, 46.5 and 55 per cent. Strains lysed by phages of both Groups I and III represented 20.6 per cent of the cultures from patients and 19.7 per cent of cultures from the personnel, while the incidence of cultures from patients and personnel that were lysed by phages of Group III and some miscellaneous phages was 9.5 and 11.3 per cent, respectively. Wise, Cranny, and Spink[9] reported that the incidence of Group I strains among the patients was 1 per cent, and in the staff, 7.3 per cent; of Group II, 2 and 8.7 per cent, respectively; and of Group III, 56.2 and 52.9 per cent.

It is sometimes possible to demonstrate that a strain of a specific phage pattern predominates in cultures from lesions, carriers, and the hospital environment. For example, Barber, Hayhoe, and Whitehead[13] found that 57 per cent of the nurses in a maternity unit were carriers of staphylococci of phage type 52A, while 58 per cent of all staphylococcal infections occurring in the newborn infants in that unit were due to staphylococci of the same phage type. Of these infections, 80 per cent were caused by antibiotic-resistant strains. Colbeck[15] reported that, coincident with an explosive outbreak of neonatal infections and breast abscesses in a hospital, the carrier rate of staphylococci of a specific phage type in the babies rose sharply from 22 to 58 per cent. The same strain was responsible for 74 per cent of the infections in babies and for 96 per cent of the breast abscesses that occurred among the mothers. Cultures of staphylococci that were isolated by Felsen and his associates[16] during an episode of staphylococcal infections in newborn babies were phage-typed in the author's labora-

tory. Approximately 60 per cent were found to be of phage type 52A. These included strains of *Staphylococcus aureus* isolated from the lesions, from the noses of babies and nurses, from a crib in the nursery, and from a sample of baby oil.

Even in the absence of any unusual number of staphylococcal infections in the nursery, Barber and her associates[17] found that staphylococci of phage type 52A were more numerous than any other type, being harbored by 37 and 34 per cent, respectively, of the nurses and babies who were carriers of staphylococci. The same strain was also isolated on occasion from the gowns of nurses who were working in the nursery. Rountree and Barbour[18] reported that strains of identical phage types were carried by the babies and nurses, and were also present in the air of the nursery.

It has been the author's experience, particularly during the past 3 years, that a large proportion of the Group III strains that have been isolated in the diagnostic laboratory have been resistant to both penicillin and the tetracyclines, and have been represented by about 3 recurring phage patterns, with some minor variations. This would appear to suggest that a limited number of strains have become established in this hospital community.

There is a direct relationship between the carriage of antibiotic-resistant staphylococci and the length of stay in the hospital environment. Barber, Hayhoe, and Whitehead[14] found that less than one fifth of the nurses harbored penicillin-resistant staphylococci upon their arrival, but that the carrier rate of those who had worked in the hospital for 3 months or more was 66 per cent. Barber and Whitehead[19] reported that only 18 per cent of the patients harbored penicillin-resistant staphylococci during the first week or less of their hospitalization while, among those who had been in the hospital for 2 weeks or more, the incidence of carriers of resistant strains was 55.5 per cent. Similarly, Barber and her associates[17] reported that among mothers in a maternity unit the proportion of carriers of penicillin-resistant staphylococci increased from 10 to 58 per cent during their 10-day stay in the hospital. This was paralleled by a rise from 6 to 35 per cent of carriers who harbored the predominating strain of phage type 52A.

It is of interest that both Colbeck[16] and Jackson and his associates[7, 20] have noted that upon the return home of a patient who had acquired a staphylococcal infection in the hospital, the strain of a given phage type was transferred to other members of the household and sometimes was responsible for infections among the family contacts.

While the preponderance of antibiotic-resistant staphylococci in cultures from hospital sources indicates their wide distribution in this environment, the evidence supplied by phage typing suggests that not only have resistant strains become established in hospitals, but these strains probably are limited to a relatively few phage types. With the gradual elimination of antibiotic-sensitive strains there is set up in the hospital a reservoir composed of resistant strains that are transferred from person to person, are present in the environment, and represent an ever-present potential source of cross-infection.

There is little doubt that staphylococci sometimes can acquire resistance to certain antibiotics during the course of antibiotic therapy. Thus, Rountree

and Thomson[21] reported conversion to streptomycin-resistance during treatment with that antibiotic in 6 instances. Wise, Cranny, and Spink[9] observed the development of resistance to erythromycin in 3 patients, and very recently we have noted the development of resistance to the tetracyclines in cultures from a patient who had received terramycin for a period of 3 weeks. In all of these cases the identity of the resistant strain with the original sensitive-infecting strain was demonstrated by their identical patterns of lysis.

The emergence of a resistant strain in cultures from a lesion that had originally yielded a sensitive *Staphylococcus*, however, is not necessarily evidence that the original strain had acquired resistance during therapy. The author has found in a number of instances that the original sensitive strain was eliminated during antibiotic therapy and was replaced by a new resistant strain of entirely different phage type. When this happened it usually occurred in a lesion that had required surgical intervention. Significantly, the new strains have exhibited phage patterns that are characteristic of the established hospital reservoir of resistant staphylococci.

While it probably is not true of all forms of staphylococcal infection, there are 2 or 3 clinical manifestations of staphylococcal disease in which the responsible strains appear to be largely restricted to a few phage types. One is acute, fulminating staphylococcal pneumonia following influenza. Williams, Rippon, and Dowsett[1] have reported that staphylococci of Group I were isolated in 56 per cent of cases of acute staphylococcal pneumonia in 2 different epidemics of influenza in England. Rountree and Freeman[22] have stated quite recently that in several cases of fulminating pneumonia during an influenza epidemic in Australia only staphylococci that were lysed specifically by phage 80 were encountered. Phage 80 was identified in late 1954 and is said to belong to Group I.

Many outbreaks of staphylococcal food poisoning are due to staphylococci of Group III. Strains of this group are responsible for the majority of outbreaks in Britain.[1] It should not be assumed that the responsibility of Group III staphylococci for food poisoning is necessarily universal, but it is interesting to note that Saint-Martin and his associates[23] found that the staphylococci incriminated in several outbreaks in Canada were of Group III, and that in the Sudan an outbreak due to staphylococci of Group III was reported by Drysdale.[24]

It may be noted that Jackson and his associates[5] found that strains of staphylococci isolated from fecal specimens in cases of enteritis following antibiotic therapy were largely of Group III. Surgalla and Dack[25] have demonstrated that staphylococci from such cases produce enterotoxin. They suggest that enteritis that follows antibiotic therapy may result from the combination of the suppression of the normal intestinal flora and the chance presence of an antibiotic-resistant enterotoxigenic strain of *Staphylococcus*.

The production of breast abscesses in adults and of staphylococcal infections in newborn infants appears to be restricted to strains of rather limited phage types.[14-18, 26, 27] Often these strains belong to Group I, but this is not always the case. Outbreaks that involved 4 hospitals in Winnepeg, Canada within a period of a few months were reported by Colbeck[15] to be caused by staphylo-

cocci that were lysed specifically by a phage "W" that he had isolated in his laboratory and that probably is related to Group III. In an outbreak investigated by Denton and his associates,[26] the phages producing lysis of the responsible staphylococci were predominantly of Group III. In the majority of reported observations, however, the staphylococci isolated from breast abscesses and neonatal infections have been lysed specifically by phage 52A.

Some of these studies can be summarized as follows. In the presence of an outbreak of staphylococcal infection in the nursery, a strain of a specific phage pattern often is found to predominate in cultures from the lesions, is present in high incidence and in similar proportions in the noses of babies and nurses, and may be found in the air or dust of the nursery, or on the furniture. Although the mothers may harbor staphylococci in their noses, their carrier rate of the specific strain is considerably lower than that of the nurses and babies. When breast abscesses occur in the mothers, the infecting *Staphylococcus* usually is found to exhibit a phage pattern identical with that occurring in the babies and nurses. During periods of little neonatal infection the incidence and phage patterns of staphylococci that are carried by the nurses and babies are closely similar, and are different from those found in the mothers. When the staphylococci carried by the individual mothers and their babies are compared, a considerable diversity is found. Rountree and Barbour[9] reported that among 54 pairs of mothers and babies, in only 7 (13 per cent) were the phage types of the staphylococci carried by mother and baby the same. In a study reported by Barber and her associates,[17] only 3 of 111 pairs carried staphylococci of identical phage patterns. In the remainder, either the baby carried staphylococci and the mother did not, or the mother harbored staphylococci and the baby's cultures were negative, or the staphylococci carried by both mother and baby were of completely different phage types.

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Discussion of the Paper

E. T. Bynoe (*Bacteriological Laboratories, Laboratory of Hygiene, Department of National Health and Welfare, Ottawa, Ont., Canada*): I should like to draw attention to a new phage type of *Staphylococcus* designated 81, discovered in our laboratories, that has proved to be one of the commonest hospital strains in Canada. This type seems to be particularly prone to produce boils and abscesses. It is not identical with, but is very similar to, type 80 reported by Rountree and her co-workers as causing several recent outbreaks of staphylococcal infection in hospitals in Australia. I am sure that this strain does not recognize the Canadian-United States border, and that it is probably widely distributed in the latter country.

Regarding the relationship of a particular *Staphylococcus* type to some special syndrome, the recent reports from England by Spittlehouse,[1] Parker, Tomlinson, and Williams,[2] and Barrow[3] would seem to give special significance to type 71 as the etiologic agent of impetigo contagiosa.

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GENETICS OF ANTIMICROBIAL RESISTANCE

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If the science of microbiology is to continue the remarkable advances that have characterized its recent development, it is essential to establish concepts and to design experiments that lead forward, rather than look to the past. This does not deny the importance of history. Rather it implies a responsibility to avoid the statement of arguments that cannot be finally resolved without new evidence from the laboratory, or that have been established in the literature long enough for every interested person to learn of the issues and the data. Accordingly, a review of reviews about the genetics of drug resistance would be of little interest to those already informed. In particular, it is not proposed to explore except briefly the overdiscussed problem of genetic versus physiological adaptation as contributing factors in the origin of drug resistance. Recent and detailed surveys are readily available.[1, 2] Instead it will be accepted provisionally that the origin of most, although perhaps not all, of the antibiotic resistance encountered in *Micrococcus pyogenes* is preceded by changes in the intracellular determinants of heredity the genes. This assumption is based partly on analogy with *Escherichia coli*, a bacterial species relatively accessible to the genetic analysis of drug resistance.[3] The ability to undergo genetic changes of significance in evolutionary adaptation is presumably a property of all living things, including bacteria.

Variability of Micrococci

Considering the large number of genetic loci that even the most primitive organism must possess a number whose magnitude is not exactly known—the investigator of microbial evolution can draw several speculative conclusions concerning the development of bacterial resistance to antibiotics: if the number of genes in *Micrococcus pyogenes* is established by conjecture at 10^3 to 10^4 (at least 1 per enzyme), and the average mutation rate is set at 10^{-5} per locus, then even a single bacterial colony consisting of several million cells must contain a variety of genetic types arising from mutations for the most part at different loci. Pleiotropy would tend to introduce some variation into the drug-resistance level of mutant strains of many types. Furthermore, since the *in vitro* development of resistance to penicillin is at least continuously variable, if not stepwise, more than 1 gene appears to be involved in providing maximum survival value in a Petri dish or test tube. Some variation could then depend on which genes are first selected in the sequence leading to full resistance. Many opportunities exist for individual differences at the cell level.

In a clone of micrococci grown from 1 or a few sensitive cells, how many mutants will arise with potentially increased resistance against a particular antibiotic? More generally, what is the likelihood that populations of pathogenic micrococci grown in the laboratory and proliferating in the animal host, or ex-

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isting in the environment, will contain cells already resistant or potentially resistant to antibiotic agents? Will the resistant cells all be alike, or are naturally occurring drug-resistant strains genetically distinct from those arising in the laboratory? These questions lead to numerous considerations, and they require a perspective somewhat broader than can be attained by confining attention to *Micrococcus pyogenes* alone. The organizers of the conference on which this monograph on staphylococcal infections is based have provided a rich field for the clinician and epidemiologist, but have simultaneously placed microbiologists concerned with heredity in a distressing position. The micrococci, in common with the mycobacteria and most other pathogens, are poor subjects for studies of bacterial heredity, their insufficiencies arising from lack of known means for cell-to-cell exchange of genetic information through sexual crossing or related phenomena. Admittedly, too little genetic exploration has been done to make possible a definitive statement. Unless some members of some *Micrococcus pyogenes* populations are partially or completely diploid at some phase of growth and reproduction, a truly precise approach to the heredity of this organism is excluded by present techniques.

Despite such limitations, or perhaps because of them, the working hypothesis that phenotypic variability is limited by genetic variability may be borrowed from research with higher organisms. Genotype evidently determines the pattern of phage resistance, a relatively constant and easily quantified characteristic. More difficulty is encountered in the study of strain differences involving production of pigment, coagulase, α-hemolysin, hyaluronidase, or any other property exhibiting continuous variation. The role of genotype as a limiting factor in the synthesis of penicillinase has not yet been defined for *Micrococcus pyogenes*, although strain differences are evident.[4, 5]

In brief, we know very little about the relative significance of genetic and nongenetic factors in the variability of staphylococci. Assuming that both types of factors contribute to variation, a long range view holds that the increased drug resistance presently encountered in clinical practice is the result of genetic variation and gradual selection. Imposed on the genetic differences is a possibility of nongenetic change in response to cultural conditions. If these physiological changes are rapid, *in vitro* experiments may provide insufficient information about transient resistance or virulence mechanisms of significance in the host. The variability of staphylococcal strains with reference to properties deemed contributory to virulence has been stressed by Lack and Wailling.[6] Distinction between genetic and nongenetic differences has more than academic interest because the former tend to be more permanent and to set the limits of fluctuation. The criterion of mere transmissibility does not distinguish between genic and extragenic changes.

With occasional attention to alternative interpretations, the following generalities will be employed in considering the development of drug resistance by bacteria:

(1) Mutations lead to differences in antibiotic sensitivity, either directly or by providing variations in the capacity for physiological adaptation through induced enzyme synthesis or otherwise.

(2) The establishment and maintenance of resistant strains is primarily a function of selection pressure.

(3) Evolutionary fitness in response to selection is reflected in the net reproductive and survival rate of entire genotypes as tested against the total complex of environmental factors. A drug-resistant cell may fail in competition with less resistant individuals in the same moderately inhibitory environment because it is of reduced virulence.

(4) Resistant cells isolated from clinical material may have their origin considered from the point of view of the patient as endogenous (derived from sensitive cells *in situ*) or exogenous (acquired from carriers or from the environment).

(5) Although the increased resistance to antibiotics noted in certain strains may depend on genetic variation, there is no corresponding requirement for identity of residual genotype or similarity of general physiological behavior beyond the *sine qua non* necessary for the mechanism (or mechanisms) of resistance. Even the spatial arrangement and location of genes contributing to multifactorial resistance tend to differ in separately isolated mutant strains.[3, 7]

Potential Limits of Mutation

Some of these generalities will now be considered more completely. Mutations leading to differences in antibiotic sensitivity have too often been considered in terms of limitless, rather than limited potentiality. The capacity of a bacterial population to become resistant is of utmost importance and is presumed to depend on the individual genes possessed by the pathogen. The geneticist maintains that bacterial species (being bacteria) have many genes in common. Some genes establish relatively higher levels of resistance *a priori*, as in the resistance of *Micrococcus pyogenes* to polymyxin. Others provide for the possibility that resistance will arise in sensitive populations by mutation at one or several specific loci. Definitions of the gene are at present operational, rather than biochemical, and they include the concept of mutation rate, or fixed probability of change, as an important property of each locus. The fact that many different genera of bacteria mutate to streptomycin resistance at a rate of about 10^{-9} suggests that the same gene may be present in these species and that related biochemical alterations are important in the provision of nonsensitive metabolic pathways. The important point is that the potential capacity of sensitive cells to mutate toward drug resistance is a function of their existing genetic structure. The chemical instabilities inherent in genetic determinants of the cell permit, not an infinite variety of mutations, but a more restricted number, which occur with definite frequencies at determinable loci. Some species of bacteria may accordingly remain sensitive to an antibiotic not only because the genes necessary to afford resistance are absent, but because the genes that might potentially mutate to resistance are also absent. Such species could be expected to differ in biochemical properties from inherently or potentially resistant species. We have neglected complications introduced by the residual genotype, and also the possibility of long range evolution from nonpotential to potential genetic adaptation. In simplest form, some bacterial species may lack, quantitatively or qualitatively, the genes necessary to acquire resistance through mutation—others may not.

In contrast with many other species of microorganism, staphylococci either possess the capacity to develop penicillin resistance or they may, in some instances, be resistant as found in nature.

Establishment of Resistance

Another generality will now be considered. If the establishment and maintenance of resistant strains are primarily functions of selection pressure, then an important question for the future concerns the long-range influence of relatively low doses of antibiotics — particularly penicillin — in regimens of mass prophylaxis. In attempts to prevent upper respiratory infection of streptococcal origin and to avoid periodic episodes of rheumatic fever, large numbers of people are being given penicillin in quantities adjusted to provide a continuously effective level.[8] At present, the emergence of resistant variants of *Micrococcus pyogenes* is believed to be favored by high-dosage regimens of penicillin and is not yet an obvious problem in the usual prophylactic program of continuous and moderate administration. Whether an eventual appearance of resistant strains will retard the progress of preventive medicine may depend on the relation between drug level and resistance level. Thus it is known that at low levels of penicillin the average resistant clone that can be recovered will have a correspondingly low degree of resistance. High concentrations of drugs more commonly allow isolation, if survivors are present, of the more extreme resistant strains.[9]

Two interpretations prevail. The first maintains the existence of a physiologically adaptive adjustment of the organism to its toxic environment. Adaptation to extreme resistance requires the evoking stimulus of a suitably high drug level. The organism adjusts to its environment and goes no further. If a potential pathogen has been held in check through prophylactic measures, it would be assumed that the necessary physiological adaptations for survival are not within the capability of the organism and would not be likely to develop in the future. The result is a philosophy of *status quo* for, except within the minor limits allowed by nongenetic variability, no opportunity exists for selection and evolution of increasingly resistant populations without recourse to elaborate theories of progressive enzymatic readjustment whose existence, however plausible theoretically, have never been incontrovertibly established.

The second point of view postulates the importance of genetic variability, *including* such manifestations of variability as have to do with physiological adaptations. Here the population of cells present at prophylactic penicillin concentrations is more diverse. It may contain an indeterminate number of mutants, unable or rarely able to survive continuous prophylaxis, yet more resistant than the sensitive prototype. If *highly* resistant cells are not present they could be expected to eventually arise from those of intermediate resistance, for example, as through exponential interaction and stepwise increase of resistance level attending an accumulation within single cells of small and individually less effective mutations. Demerec[10] has shown that the development of penicillin resistance in *Micrococcus pyogenes* var. *aureus* requires consecutive exposure of increasingly resistant survivors to progressively higher levels of the

antibiotic. It has not yet been determined whether prophylaxis will eventually lead to similarly increased resistance *in vivo*.

Reproduction and Survival of Mutants in the Host

Failure of resistance to appear in animal hosts could depend on the inability of resistant mutants to maintain the necessary virulence or survival value for successful existence. Selection would favor strains able to become resistant without becoming avirulent. Establishment or nonestablishment of mutant bacterial strains, even *in vitro*, is related to complicated environmental factors, including in a selected example the effects of metabolites produced by bacteria themselves as shown in the elegant experiments of Braun and his colleagues.[10] Phenotypic variations of significance in the pathogenicity of penicillin-resistant mutants of *Micrococcus pyogenes* might include differences in production of coagulase, staphylokinase, hyaluronidase, hemolysins, penicillinase, and various undetermined substances conditioning survival in human blood or serum. Changes of possible selective implication are often associated with antibiotic resistance, such as slow growth (G forms)[11, 12] alterations in response to Gram's stain[13, 14] and modifications in the level of the required amino acid.[15] Antibiotic resistance may be offset by correlated alterations producing negative selection in human fluids and tissues. Resistance must be considered as only one facet of the genetic constitution required for survival in the presence of antibiotic.

Endogenous and Exogenous Derivation of Resistant Strains

The occurrence of penicillin-resistant staphylococci in hospitals, as reported by epidemiologists and clinicians, is not consistent with the concept of gradual selective changes occurring within a patient at rates more or less proportional to the intensity of selection pressure.

For one thing, strains of intermediate resistance as observed in the laboratory are relatively rare in infections,[16] although it should be remembered that transitional states may be illusive because of briefness. Furthermore, when sensitive and resistant strains are recovered from the same patient they frequently give different phage patterns, as though unrelated.[17] Investigations described by several contributors to this monograph prove with the phage-typing technique that patients in the surgical wards of hospitals do not develop their own penicillin-resistant strains by mutation, but acquire them as secondary infections from carriers, predominantly among the hospital personnel. An important question is this: How far back must one trace the resistant strain of a carrier before coming upon a mutational origin from sensitive cells? This problem has not been adequately investigated. The tendency for different hospitals to be repositories of different penicillin-resistant strains suggests, as do laboratory studies, that penicillin resistance can arise in strains of any phage pattern.[18] Concentration of antibiotic-resistant strains in the group III of Williams and Rippon has led to the suggestion by Barber and Whitehead[17] that this group is genetically unstable. A more recent explanation by Gould[19] is that sensitive nonpenicillinase producers of Groups I and II may give rise by mutation to

both slow-growing nonpenicillinase-producing resistant colonies of the same phage pattern *and* fast-growing penicillinase producers of type III. Independent corroboration would be reassuring, particularly since some bacteriologists would regard the appearance of a new phage pattern as evidence of contamination *in vitro*, just as it is deemed indicative of contamination or cross infection in the hospital.

Unless bacteria are well-isolated in the infected tissue it does not appear probable that the critical conditions essential for laboratory isolation of resistant strains would be duplicated in animal hosts. *In vitro* experiments reveal that if strains of extreme resistance to penicillin are to be obtained with maximum efficiency, it is necessary to provide either a gradient concentration of the antibiotic or a carefully adjusted series of steps leading to increased antibiotic levels. An essential part of the process is provision of intervals wherein the most resistant elements of a bacterial population will be able to undergo extensive multiplication. On the average, large numbers of bacteria are required to give rise to further mutations. In individuals receiving massive doses of penicillin an exogenous source of resistant strains appears more probable, since a suitable gradient to low concentrations may not pertain. There is also considerable uncertainty about the relationship between penicillinase-producing resistant strains responsible for animal infections and the kind of resistant organisms isolated by selection in the laboratory.

Artificial and Natural Resistance

Inability of penicillinase-producing strains of *Micrococcus pyogenes* to become established after their origin as mutants in laboratory cultures led formerly to the view that such types could be found only in nature. Micrococci able to grow in the presence of penicillin were divided into "artificial" or nonpenicillinase-producing types, arising by stepwise mutations only in the laboratory, and "natural" or penicillinase-forming strains, never found in mutation-selection experiments.[20] Szybalski[21] demonstrated, however, that a substance able to inactivate penicillin is indeed produced by a rare type of mutant arising from sensitive populations of *Micrococcus pyogenes* var. *aureus* exposed to this antibiotic by the gradient plate procedure. Difficulties in isolation exist because the individual cells produce relatively small amounts of penicillinase and must therefore grow in mutual contact, as in a colony proliferating at low concentrations near the inhibitory threshold on a gradient plate.

Szybalski has stated that the presumptive penicillinase-producing mutants isolated by the gradient plate procedure "retain growth and fermentation characteristics as well as virulence unchanged," and infers a similarity between them and the so-called natural type commonly found in animal infections. Somewhat parenthetically, it is admitted that penicillinase producers derived directly *in vitro* from the sensitive population by mutation may be less well-adapted than what we may now be forced to call the natural "natural" types. The latter have had prolonged evolutionary testing, and represent the most successful survivors of a large number of prototypes. The production of coagulase by these natural strains may be understood as adaptively valuable,

but their superiority as penicillinase producers presents something of a puzzle for encounters with penicillin in effective concentrations are comparatively recent on the time scale of microbial existence. Perhaps penicillinase has some value to the commonly isolated pathogens that is independent of its usefulness for survival in the presence of the antibiotic. On the other hand, metabolic products that distinguish the average naturally occurring pathogen (for example, coagulase and α-hemolysin) may define a general metabolism in which the production of penicillinase is favored. Without further evidence, it would seem unwarranted to classify penicillinase producers arising by mutation *in vitro* as equivalent to wild type-resistant strains. Explicit experiments on the virulence of such mutants would be of great interest. The presence or absence of penicillinase provides a convenient classification, but either condition should not obscure the fact that a mutant is always more similar in its residual genotype to the strain from which it arose than to a distantly related form possessing some common enzyme or superficial property.

Uniqueness of Resistant Strains

A number of investigators, including Eagle,[4] have stressed the existence of several types of penicillin-resistant staphylococci. The experience derived from bacterial genetics shows that a single genetic locus is involved in resistance to streptomycin.[22] Even here, individual mutants tend, if inspected by certain criteria, to be dissimilar and perhaps even unique.[23] Where several genes are required for resistance, as is probable in the example of penicillin-resistant staphylococci, the opportunity for individual differences would be even greater. The final generality, stressing the opportunities for variation in residual genotype and in physiological behavior, introduces uncertainty into inductive conclusions about staphylococci as a group. If resistant strains in one laboratory are genetically different from those in another, and if all have the capacity for superimposed physiological change, then the search for "common" factors of resistance and virulence may continue to prove illusive.

Conclusion

Without relinquishing the well-established evidence that antibiotic resistance can arise by mutation, it is necessary to investigate more extensively the time intervals and diverse evolutionary changes that may intervene between sensitive progenitors and resistant descendants. An understanding is required that selective fitness may be based on both genetic and nongenetic variation, with drug resistance as only 1 element of fitness. Some useful analogies may be drawn from comparisons with species of bacteria that undergo genetic recombination. Antibiotic resistance in staphylococci, however, is undoubtedly complex, and it is not yet amenable to precise genetic analysis. The genetic tools are presently too few, and the varieties of *Staphylococcus* are too many.

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SMALL COLONIES (G VARIANTS) OF STAPHYLOCOCCI: ISOLATION FROM CULTURES AND INFECTIONS

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Minute colonies have been isolated from strains of various genera of bacteria by many investigators following exposure of cultures to adverse environmental conditions such as chemicals, bacteriophages, prolonged incubation, storage, and antibiotics. Similar colonies have also been observed in cultures of material from septic processes in humans and animals. Previous reports of the characteristics of small-colony variants have been controversial and incomplete, and the role of these variants in infectious processes has remained unknown. A review of the literature regarding these aberrant colonies has been presented by Wise and Spink.[1]

The small colonies are called "G" (gonidial) colonies following the designation of Hadley, Delves, and Klimek[2] in 1931, who observed them after culturing *Shigella* in bacteriological media containing lithium chloride. G colonies have a diameter of less than 0.1 mm., and some strains are discernible only with magnification. They revert to normal larger colonies when subcultured on nutrient media.

Selection of G Colonies of Staphylococci with Antimicrobial Agents

Hoffstadt and Youmans[3, 4] and Swingle[5] isolated small-colony forms of *Staphylococcus aureus* from cultures in 0.5 per cent lithium chloride in beef infusion broth. In other reports, Youmans[6] and Youmans and Delves[7] studied the effect of inorganic salts on staphylococci and found that barium chloride and barium nitrite selected dwarf colonies in peptone water. Hale[8] subcultured G variants from broth containing barium chloride and reported the small colonies to be about fourfold more sensitive to penicillin than the parent strain.

In 1943 an antibiotic was found to be effective in the selection of G colonies by Schnitzer, Camagni, and Buck[9] who observed minute translucent colonies in areas where growth of staphylococci had been inhibited by a crude filtrate of a culture of *Penicillium notatum*. The G colonies reverted to larger, parent types when transferred to fresh media containing no antibiotics, but reversion was prevented by cultivation in media that contained filtrates of a culture of the fungus. The G cultures were more resistant to the inhibitory action of the metabolic products of the fungus than were the parent strains. Later, Youmans, Williston, and Simon[10] isolated small-colony variants from 9 of 10 strains of staphylococci by cultivation in penicillin broth. Rapid reversion of the G cultures, however, prevented testing of the susceptibility of the strains to the antibiotics. Eriksen[11] produced small colonies of staphylococci by serial transfer in broth containing increasing concentrations of penicillin. Two strains showed pure cultures of G colonies, but reversion to large colonies occurred after transfers were made in plain broth. Purity of G colonies was maintained in subcultures in penicillin broth. Stable dwarf colonies of staphylococci were

obtained from broth containing streptomycin, penicillin, and other inhibitory substances by Browning and Adamson,[12] who reported the colonies to have a reduction in hemolysin production but retention of coagulase. Szybalski,[13] using an agar-diffusion method with penicillin, observed small-colony variants as satellites around large colonies. Barbour[11] found that G colonies appeared in 5 cultures during the *in vitro* development of resistance to streptomycin. The variant colonies were of pin-point size, nonpigmented, and nonhemolytic.

Characteristics of G Variants Selected with Antibiotics

Wise and Spink[1] have reported studies of G cultures of *Staphylococcus* isolated with the aid of the antibiotics, penicillin, erythromycin, carbomycin, and bacitracin. Attempts to select the variants with streptomycin, tetracycline, and chloramphenicol, however, failed with the concentrations of antibiotics that were employed. The colonies were translucent, nonpigmented, and had diameters of less than 0.1 mm. Reversion to large colonies occurred, however, when the strains were cultivated in the absence of antibiotics, but the rate of reversion varied in that large colonies appeared in some of the strains in 24 hours and others were stable when transferred daily for 1 month. Minute colonies in the presence of larger, reverted colonies of normal size are demonstrated in FIGURE 1. The morphology of the G cells did not differ from those of the

FIGURE 1. Showing comparative size of many G colonies and 4 larger reverted colonies of staphylococci of normal size.

normal or reverted bacteria, with the exception of a few large swollen cells in some of the G strains.

Gladstone's[15] synthetic medium supported growth of the parent and reverted cultures, but the G cultures showed no growth in this medium. There was no evidence of production of hemolysin or coagulase. Penicillinase could not be demonstrated. The G cultures obtained with penicillin, however, were twofold to eightfold more resistant to this antibiotic than were the parent strains. Penicillinase was not produced by G cultures adapted to grow in concentrations of penicillin up to 2,000 units per ml. in broth, and the acquired resistance to penicillin was lost with reversion to large colonies.

The use of 34 bacteriophages in the typing of 15 sets of related strains demonstrated a genetic relationship of the parent, G, and reverted cultures, thereby eliminating the possibility that the G or reverted strains were contaminants. The G cultures were not susceptible to lysis with diluted bacteriophages as were the parent and reverted strains but required a greater concentration of bacteriophage for lysis. When reversion occurred there was a return to a lytic pattern identical with that of the parent in 14 of the 15 strains, and similar in 1 set of cultures.

The parent and reverted strains were identical in all characteristics studied, with the exception of their stability in the presence of subinhibitory concentrations of the antibiotic that had previously been used in their selection. Dwarf cultures could be readily produced from reverted cultures by employing the tube-dilution method commonly used for determining the *in vitro* susceptibility of bacterial cultures to antibiotics. After 24-hour incubation with this method, subcultures from the test tubes with the greatest concentration of antibiotics, in which no bacterial growth was visible, showed the presence of G colonies.

A comparative study of the virulence of the related variants was made by injecting standardized suspensions of the parent, G, and reverted bacteria intradermally into rabbits and intravenously into mice. In rabbits the sites of intradermal injections with G cultures were just discernible after 48 hours, whereas edema, erythema, pustule formation, and necrosis appeared following injection of the parent and reverted strains. The reverted strains produced lesions of similar or greater severity than the reaction produced by the related parent strains.

The G cultures were avirulent when injected intravenously in mice, as demonstrated by 100 per cent survival, although G cultures could be isolated from the kidneys of apparently healthy mice when sacrificed 2 weeks after inoculation. Conversely, all mice that were injected with the parent and the reverted cultures were dead in 120 and 72 hours, respectively, with death occurring at a more rapid rate in the mice infected with the reverted cultures.

The Isolation of G Cultures from Clinical Infections

The primary isolation of G cultures of staphylococci from human sources is of considerable clinical interest because little is known concerning the role of these bacterial variants in disease. In 1951 Hale[9] first reported the isolation of G colonies in pure culture from an abscess and also obtained similar colonies in a

culture from the patient's nose. The variant cultures grew as normal large colonies in the presence of 10 per cent carbon dioxide. There was no report of therapy in this patient. Sherris[17] also found pure cultures of G colonies of *Staphylococcus* from closed abscesses in 2 patients who had received penicillin for 24 hours prior to the collection of material for bacteriological examination, and he confirmed Hale's observation of increased size of colonies during incubation in carbon dioxide. Wise and Spink[1] isolated 8 G cultures from 7 patients. Six of the patients had received antimicrobial therapy prior to isolation of the small-colony variants. All of the strains showed gradual reversion to colonies of larger size on subculture. Recently, Goudie and Goudie[18] reported the isolation of multiple pure cultures of stable dwarf colonies from the nose during recurrent infections of a patient over a period of 7 months. Also, Thomas and Cowlard[19] describe a strain of G colonies of bacteriophage type 52A isolated from infections of 2 members of 1 household, 1 of whom had been treated with penicillin. This would indicate that G cultures are transmissible and may be primary pathogens. A summary of reported cases is presented in TABLE 1.

Cultures containing many G colonies have been isolated from infections in 2 patients at the Jefferson Medical College Hospital, Philadelphia, Pa. The first patient, a 57-year-old colored female, was admitted January 18, 1956, with a necrotic infection of the left hand that occurred following treatment of a paronychia of the left index finger with hot applications that caused severe burns of the hand. The patient received no antimicrobial therapy prior to bacteriological studies. A culture of the necrotic material contained a mixture of G colonies and large colonies of staphylococci. The second patient, a 62-year-old male, was admitted to the Barton Division of the Jefferson Medical College Hospital on January 27, 1956, with emphysema, chronic pulmonic disease, and a cough that was productive of purulent sputum. He had received penicillin,

TABLE 1
THE ISOLATION OF G CULTURES FROM SEPTIC INFECTIONS OF PATIENTS

No.	Source	Authors	Treatment
1.	Abscess and nose	Hale[16]	No report
2.	Abscess	Sherris[17]	Penicillin (24 hours)
3.	Abscess	"	Penicillin (24 hours)
4.	Pleural fluid	Wise & Spink[1]	Penicillin
5.	Urine	" " "	Sulfisoxazole
6.	Throat	" " "	Penicillin Streptomycin
7.	Urine	" " "	Chlortetracycline
8.	Urine	" " "	Sulfisoxazole Chlortetracycline
9.	Blood	" " "	Penicillin
10.	Urine	" " "	None
11.	Abscesses and nose	Goudie & Goudie[18]	Penicillin
12.	Abscess	Thomas & Cowlard[19]	Penicillin
13.	Abscess	" " "	None
14.	Abscess	Wise[20]	None
15.	Sputum	"	None

streptomycin, and tetracycline for 5 days approximately 5 months prior to admission. Bacteriological examination of his sputum revealed both G colonies and large colonies of staphylococci. The G colonies from both patients grew as large colonies during incubation in 10 per cent carbon dioxide. Aerobic subcultures of the large colonies that had developed in carbon dioxide resulted in a return to the small-colony type.

Discussion

Small-colony G variants have been isolated from bacterial cultures under *in vitro* experimental conditions that were unfavorable for bacterial multiplication. Cultures of staphylococci revealed G colonies when cultivated in the presence of antibiotics in concentrations that were inhibitory but not rapidly bactericidal for the original strains. G cultures have been isolated from patients after therapy with antibiotics. Similar colonies have been obtained from patients who had no recent antimicrobial therapy.

It was demonstrated that G cultures produced *in vitro* with the aid of an antibiotic were twofold to eightfold more resistant to the antibiotic than were the parent strains. They were devoid of hemolysin, coagulase, and penicillinase activity. The G variants lacked virulence and remained viable in animal tissues without producing signs of infection. Reversion *in vivo* to cultures of large colonies has not been proved. The large, reverted colonies were similar to the parent colonies in appearance, production of hemolysin and coagulase, susceptibility to antibiotics, and virulence for experimental animals, but were different in that G cultures were obtained more readily from the reverted cultures than from the parent colonies.

Small-colony variants isolated from human infections also reverted to large colonies when subcultured in nutrient media. They appeared as large colonies when incubated in 10 per cent carbon dioxide. Comparative studies of G cultures isolated from humans and those obtained *in vitro* with the aid of antibiotics have not been successful because reversion has made such investigations difficult.

It may be possible, with optimum concentrations of antibiotics, for the survival of G cultures to occur in human tissues during therapy of staphylococcal infections. Under these conditions they may remain undetected and later revert to virulent forms, with relapse of infection. Investigations of this possibility are in progress. Careful examination should be made in the bacteriological investigation of infectious processes for the presence of these small colony variants.

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THE CLINICAL PROBLEM OF ANTIMICROBIAL RESISTANT STAPHYLOCOCCI*

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A publication devoted to *Micrococcus pyogenes* and the responses of the invaded host is timely because of the serious problem that antibiotic-resistant staphylococci poses for the clinician. No attempt will be made to review the extensive literature on the subject, since such a summary has been made elsewhere.[1] This report will be concerned primarily with the problem of staphylococcal sepsis as it has been encountered at the University of Minnesota Hospitals and its affiliated clinics since 1937, thus permitting the development of the over-all picture of staphylococcal infections as it has been seen in one medical center.

The Appearance of Resistant Strains of Staphylococci in Relation to the Use of Antibiotics

There appears to be no question that, as antibiotics have been introduced for general use, antibiotic-resistant strains of staphylococci have appeared in those areas where each of the antibiotics has been used most extensively. This applies especially to large general hospitals. Before unfolding this aspect of the subject as it applies to institutions in the area of Minneapolis, Minn., brief consideration should be given to techniques used for determining the *in vitro* sensitivity of staphylococci to antibiotics, and to the clinical significance of the results. For both clinical and investigational purposes we have utilized the tube-dilution method for measuring *in vitro* sensitivity. Fortunately, virtually the same technique has been employed over the years of the study. Testing the action of antibiotics against broth suspensions of bacteria has given more reliable results than the rough data obtained with discs on agar media. Even when the most precise *in vitro* methods of testing are used, the results are only of relative value when applied by the clinician for the selection of proper antibiotics in human patients. It is quite evident in selecting antibiotics for therapy that indiscriminate testing is carried out too often by clinicians, and that too much reliance is placed upon the results of the tests rather than upon sound clinical judgment and good bacteriology. Nevertheless, 2 serious types of bacterial infection, in which the added information of *in vitro* bacterial sensitivity to antibiotics may be advantageous for the patient and for the clinician are subacute bacterial endocarditis and severe staphylococcal sepsis, particularly septicemia. In fact, in discussing shock due to various species of bacteria, Hall and Gold[2] have stated that the mortality rate was significantly reduced when appropriate therapy was directed by the results of *in vitro* sensitivity tests.

Referring now to the changing sensitivity of strains of staphylococci, prior to

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TABLE 1

IN VITRO RESISTANCE OF STRAINS OF COAGULASE-POSITIVE STAPHYLOCOCCI ISOLATED AT THE MINNEAPOLIS GENERAL HOSPITAL DURING 1953, 1954, AND 1955

Antibiotic	Year	Total no. of strains	Per cent resistant to >3.1 units or μg. per ml	Per cent resistant to >50 units or μg. per ml
Penicillin	1953	154	85.06	61.04
	1954	156	83.72	73.10
	1955	209	88.00	70.81
Streptomycin	1953	154	95.45	65.60
	1954	156	100.00	69.80
	1955	209	99.04	69.38
Chlortetracycline	1953	154	66.23	63.00
	1954	156	67.88	66.20
	1955	139	64.03	51.80
Oxytetracycline	1953	154	66.23	62.90
	1954	156	67.31	60.20
	1955	139	64.03	56.11
Tetracycline	1954	156	67.31	56.50
	1955	209	67.46	49.76
Chloramphenicol	1953	154	100.00	0.65
	1954	156	100.00	0.78
	1955	209	99.52	1.91
Erythromycin	1953	153	21.43	18.80
	1954	156	21.15	14.70
	1955	209	24.88	22.49
Neomycin	1953	154	4.54	0
	1954	155	5.81	0
	1955	209	6.70	0

the introduction of penicillin into the University of Minnesota Hospitals not a single culture of 67 strains of coagulase-positive staphylococci isolated from patients was found to be resistant to more than 0.8 unit of penicillin.[3] It is recognized that some investigators have isolated an occasional strain of highly resistant staphylococci prior to 1942, but these naturally-resistant strains were not encountered in our material. During 1949 and the early part of 1950, one half of the strains of coagulase positive staphylococci isolated from the patients at the University Hospitals were highly resistant to both penicillin and to streptomycin.[4] More recent data covering the years 1953, 1954, and 1955 are presented in TABLE 1. This information relates to strains of coagulase-positive staphylococci obtained from patients being treated at the Minneapolis General Hospital. Miss May Collin performed all of the sensitivity tests, employing the same tube-dilution technique that has been described elsewhere.[5] A significant feature is that about 70 per cent of the strains were resistant to more than 50 units of penicillin per ml. and to well over 50 μg. per ml. of streptomycin and to the tetracycline drugs. It is of interest that the resistance of staphylo-

cocci to erythromycin is considerably less, and that the percentage of strains resistant to this antibiotic has been fairly well stabilized over the 3-year period. About 20 per cent of the strains have been resistant to more than 50 μg. per ml. of erythromycin. Another important observation is that a very small number of strains were highly resistant to chloramphenicol during these 3 years. This finding is no doubt related to the fact that chloramphenicol was not widely used in the hospital because of apprehension concerning its toxic activity on the bone marrow. The fact that so few strains were found to be resistant to chloramphenicol made it possible for the clinicians to turn to this antibiotic when such a large proportion of strains was observed to be highly resistant to the other commonly used antibiotics. A more detailed analysis of the results of *in vitro* sensitivity tests obtained in 1955 are shown in TABLE 2, and the results with some of the newer antibiotics are also given. It is to be noted that many of the strains revealed a high degree of resistance against penicillin, streptomycin, and erythromycin. The results with cathomycin and streptonivicin parallel each other. Spiramycin does not appear to be a promising drug for staphylococcal sepsis because of the considerable degree of resistance exhibited by many strains. The potentialities of vancomycin remain to be explored.

Changing Clinical Pattern of Staphylococcal Infections

There has been a remarkable change in the pattern of staphylococcal infections as seen in the University of Minnesota Hospitals since 1937. Prior to the introduction of penicillin in 1942, staphylococcal osteomyelitis of the long bones was a common and discouraging type of infection to be found in the wards, especially on the pediatric and orthopedic services. Today, acute osteomyelitis is rarely seen in the hospital, and even chronic forms of the disease are encountered much less frequently. Carbuncles and furuncles are to be seen only occasionally in the hospitals. On the other hand, there has been a serious increase in the incidence of severe staphylococcal sepsis, with and without bacteremia, many of the cases having been acquired in the hospitals. The number of cases of acute and chronic staphylococcal infections of the genitourinary tract is considerable, and the management of these patients remains depressing for both the clinician and for the patient. The incidence of staphylococcal pyoderma in patients with debilitating or degenerative diseases remains high, particularly in the bedridden and aged patients. As will be detailed shortly, staphylococcal sepsis has become an increasingly alarming problem in the general hospitals, and this feature is directly related to the appearance of antibiotic-resistant staphylococci in abundance in such an environment.

The Changing Mortality Rate of Staphylococcal Septicemia

Prior to the advent of penicillin, the over-all mortality rate accepted by most investigators for staphylococcal septicemia was about 75 per cent.[6] Although the sulfonamides, especially sulfathiazole, appeared to offer hopes for reducing this rate, such a sanguine outlook proved short-lived with further experience. It soon became apparent, however, that penicillin was a very potent and encouraging drug for staphylococcal septicemia. By 1945 it became possible to

TABLE 2

CONCENTRATIONS OF ANTIBIOTICS REQUIRED TO INHIBIT GROWTH OF STRAINS OF COAGULASE POSITIVE STAPHYLOCOCCI ISOLATED AT THE MINNEAPOLIS GENERAL HOSPITAL DURING 1955

Antibiotic	Total no. strains	Concentration of antibiotic in units or μg per ml necessary to inhibit growth															Per cent of strains >3.1 units or μg.	Per cent of strains >50 units or μg.
		>200	1600	800	400	200	100	50	25	12.5	6.3	3.1	1.5	0.75	0.38	0.19	0.09	
Penicillin	209	1	26	50	33	19	19	11	11	5	9	5	1		1	4	14	88.04	70.81
Streptomycin	209		136	4	1	3	1	3	24	23	12	1			1			99.04	69.38
Chlortetracycline	139				39	30	3	7	7	2	1	16	33	1				64.03	51.80
Oxytetracycline	139				40	31	7		4	6	1	3	37	10				64.03	56.11
Tetracycline	209				6	36	62	19	15	3	0	1	25	39	3			67.46	49.76
Chloramphenicol	209						4	2	1	104	97	1						99.52	1.91
Erythromycin	209			8	29	8	1	1			3	2			15	122	19	1	24.88	22.79
Bacitracin	209								2			34	110	54	8			1	17.22	0
Neomycin	209									1	7	6	25	57	92	19	1	1	6.70	0
Cathomycin	108								2	2	3	2	13	26	51	9			8.33	0
Streptonivicin	71									3		1	1	12	42	11	1		5.63	0
Spiramycin	53				6			1	3	1	33	9							100.0	14.0
Vancomycin	34										1	15	18						47.06	0

report on a marked drop in the mortality rate at the University Hospitals because of the use of penicillin, the fatality rate in a treated group of cases being 28 per cent.[6] Within a few years, however, it was disheartening to realize that this improved mortality rate could not be maintained. In a group of cases treated at the University of Minnesota and its affiliated hospitals between 1951 and 1953, the mortality rate was 54 per cent, twice what it had been previously.[7] More recently, the records of 24 patients having staphylococcal septicemia at the University Hospitals from 1952 through 1955 have been analyzed in collaboration with Gloria Bradley. The pertinent data pertaining to these patients are shown in TABLE 3. Seventeen of the 24 patients expired, giving a mortality rate of 70.8 per cent. In addition to these cases of septicemia, 17 cases of acute bacterial endocarditis have been studied, and 16 of these individuals died, resulting in a mortality rate of 94.1 per cent. Information on these patients is shown in TABLE 4. If all the cases of septicemia, with and without the presence of endocarditis, are combined, there is a total of 41 cases, and 33 of these patients died, yielding an over-all mortality rate of 80.48 per cent. In other words, at the end of 1955, we find that staphylococcal septicemia is not usually associated with osteomyelitis, but its mortality rate approximates that present before the introduction of the antibiotics.

Some Factors Responsible for the Development of Staphylococcal Septicemia

We have attempted to ascertain some of the factors responsible for the development of staphylococcal septicemia in the 41 patients already referred to. This information is summarized in TABLE 5. The most disturbing feature is that the infections of at least 24 of the 41 cases, or about 60 per cent, were acquired in the hospital, and most of these patients were on surgical services. Of serious concern is the relatively large number of infections that originated from minor surgical procedures, such as "cut-downs" for intravenous therapy. In several instances a localized thrombophlebitis resulted that was the primary focus for the staphylococcal infection. It is to be noted that there were 4 cases of septicemia which were probably induced by the use of corticosteroids for chronic disease.

Relationship of Penicillin-Resistant Strains of Staphylococci to the Outcome of Patients Having Septicemia

It is generally agreed that penicillin is the most effective therapeutic agent for staphylococcal sepsis, provided that the organisms are sensitive to the drug. Unfortunately, *in vitro* sensitivity tests were not carried out on all of the strains isolated from the patients, but data are available on 27 patients. Twenty-two of these patients had infections with strains that were resistant to more than 50 units per ml. of penicillin, and 18 of the 22, or 81.8 per cent, died.

Since so many of the cases of septicemia were contracted in the hospital, and since the experience of many investigators has revealed that these hospital infections are largely due to penicillin-resistant strains of staphylococci, the present series of cases was investigated for the relationship between the out-

TABLE 3

CASES OF SEPTICEMIA DUE TO COAGULASE-POSITIVE STAPHYLOCOCCI, UNIVERSITY OF MINNESOTA HOSPITALS, 1952–1955

Age & sex	Precipitating factor	*In vitro* sensitivity to antibiotics (Units or μg. per ml.)		Therapy	Outcome
		Resistant	Sensitive		
39 F	Postoperative wound infection	Pen. >15.6	C-tet. 7.8 Chlor. 7.8 Eryth. 1.0 Bac. 0.8	Penicillin Streptomycin Chlortetracycline Bacitracin Erythromycin	Expired
44 F	Postoperative wound infection Ca of cervix with metastases	Pen. >500.0 Strep. >62.5 C-tet. 62.5	Eryth. 0.5 Bac. 1.0	Penicillin Streptomycin Chlortetracycline Neomycin Bacitracin Erythromycin	Expired Autopsy
71 M	Postoperative wound infection Uremia			Penicillin Streptomycin	Expired
38 M	Thrombophlebitis following "cut down" for I.V. infusion Lymphoblastoma	Pen. >62.5 Strep. >62.5 O-tet. >62.5	Chlor. 7.8 Eryth. 1.9	Penicillin Streptomycin Oxytetracycline Erythromycin Bacitracin	Expired
77 M	Postoperative pneumonia	Pen. >15.6 Strep. >62.5 C-tet. >62.5 O-tet. >62.5		Penicillin Streptomycin Oxytetracycline Bacitracin Erythromycin	Expired
67 M	Postoperative wound infection	Pen. >15.6 Tetra. >15.6	Chlor. 3.9 Eryth. 1.0	Tetracycline Streptomycin Erythromycin Chloramphenicol	Recovered

77 M	Abdominal surgery Perforated bowel Ca of colon			Penicillin Streptomycin Chlortetracycline Oxytetracycline	Expired Autopsy
7 F	Congenital heart disease Pre-op. tonsillitis Postop. wound infection			Penicillin Streptomycin Chlortetracycline	Expired Autopsy
42 M	Ca esophagus Postop. wound infection	Pen. >15.6 Strep. >62.5 C tet. >31.2 O-tet. >62.5	Chlor. 7.8	Penicillin Streptomycin Chlortetracycline Oxytetracycline Chloramphenicol Erythromycin Bacitracin	Expired Autopsy
74 M	Ulcerative colitis Postop. wound infection Cortisone therapy	Pen. >15.6	O-tet. 6.2 Chlor. 1.0 Eryth. 1.0 Bac. 0.8	Penicillin Chloramphenicol Cortisone	Expired Autopsy
44 F	Bowel resection for obstruction Postop. wound infection	Strep. >62.5 O tet. >15.1 Chlor. >15.1	Eryth. 0.25 Bac. 1.0 Magna. 1.0	Penicillin Streptomycin Chlortetracycline Bacitracin Magnamycin	Recovered
60 F	Ca of ovary ? Postop. wound infection	Chlor. >31.2 Eryth. >62.5	Strep. 7.8 C-tet. 7.8	Erythromycin Penicillin	Expired Autopsy
57 F	Splenectomy for Banti's disease Thrombophlebitis following I.V. "cut-down"	Pen. >15.6 O-tet. >15.6	Chlor. 7.8 Eryth. 1.0 Bac. 3.2	Penicillin Oxytetracycline Chloromycetin Erythromycin	Expired Autopsy

TABLE 3—*Continued*

Age & sex	Precipitating factors	*In vitro* sensitivity to antibiotics (Units or μg. per ml.)		Therapy	Outcome
		Resistant	Sensitive		
38 M	Tetanus Tracheotomy		Pen. 7.8 Chlor. 7.8 Eryth. 7.8 Bac. 12.5	Penicillin Streptomycin Chloramphenicol Erythromycin Bacitracin	Recovered
84 M	Thrombophlebitis	Pen. >15.6 Strep. >62.5 Tetra. >15.6 Eryth. >62.5	Chlor. 7.8 Bac. 3.2	Tetracycline Penicillin Streptomycin Chloramphenicol Bacitracin M 3339	Expired Autopsy
44 F			C-tet. 0.5 Eryth. 1.9 Bac. 6.3	Chlortetracycline Oxytetracycline Erythromycin Bacitracin	Recovered
9 F			Pen. 0.5 Chlor 7.8 Tetra. 0.25 Eryth. 1.0 Bac. 3.2	Penicillin	Recovered
37 F	Acute myelogenous leukemia Cortisone			Penicillin Chlortetracycline Erythromycin	Expired
1½ F	Congenital heart disease Tonsillitis	Pen. >15.6	Strep. 7.8 C-tet. 1.0 O-tet. 1.0	Penicillin Streptomycin Oxytetracycline	Recovered

14 days F	Pyoderma Congenital heart disease	Pen. Strep. C-tet.	15.6 62.5 62.5	Eryth.	1.0	Penicillin Streptomycin Erythromycin	Expired Autopsy
1 F	Fibrocystic disease of pancreas Bronchopneumonia					Penicillin Chlortetracycline	Expired Autopsy
1 month M	Upper respiratory infection	Pen. Chlor. C-tet.	>15.6 >62.5 >62.5	Eryth.	1.0	Penicillin Erythromycin	Recovered
58 M	Infection of toe Diabetes mellitus					Penicillin Chlortetracycline Erythromycin	Expired Autopsy
7 F	Acute myelogenous leukemia	Pen. Tetra.	>15.6 >62.5	Eryth. Bac.	1.9 3.2	Chlortetracycline Erythromycin Penicillin	Expired Autopsy

Pen., penicillin; strep., streptomycin; tetra., tetracycline; C-tet., chlortetracycline; O-tet., oxytetracycline; chlor., chloramphenicol; eryth., erythromycin; bac., bacitracin; and magna., magnamycin

TABLE 4

CASES OF ACUTE BACTERIAL ENDOCARDITIS DUE TO COAGULASE-POSITIVE STAPHYLOCOCCI AT THE UNIVERSITY OF MINNESOTA HOSPITALS, 1949–1955

Age & sex	Precipitating factors	*In vitro* sensitivity to antibiotics (Units or μg. per ml.)		Therapy	Outcome
		Resistant	Sensitive		
52 M	Ulcerative colitis Cortisone Postoperative wound infection	Pen. >15.6 Strep. >62.5 Tetra. >15.6	Chlor. 3.9 Bac. 3.2	Penicillin Streptomycin Tetracycline Erythromycin Chloramphenicol	Expired Autopsy
48 M	Gastrectomy Postoperative thrombophlebitis	Pen. >15.6 Strep. >62.5 O-tet. >15.6	C-tet. 7.8 Chlor. 3.9 Eryth. 1.0 Bac. 1.6	Penicillin Streptomycin Bacitracin Erythromycin	Expired Autopsy
54 M	? Catheterization urinary bladder Uremia	Pen. >15.6 Tetra. >62.5 Eryth. >31.2	Bac. 6.3	Penicillin Tetracycline Streptomycin Erythromycin M 3339 Hydrocortisone	Expired Autopsy
34 F	Abortion Rheumatic heart disease		Pen. 0.05 Strep. 3.12 Chlor. 3.9 Eryth. 0.5 Bac. 6.3	Penicillin	Expired Cardiac failure
70 M	Infected toe ? Rheumatic heart disease		Pen. 0.13 Tetra. 0.25 Chlor. 3.9 Eryth. 0.5	Penicillin Streptomycin	Recovered
25 F	Rheumatic heart disease	Pen >15.6 Strep. >7.8	C-tet. 3.9 O-tet 7.8 Bac. 1.0	Penicillin Streptomycin Bacitracin	Expired Autopsy

51 M	Rheumatic heart disease				Streptomycin Chlortetracycline	Autopsy
67 F		Pen. >15.6	C-tet. 1.9 Eryth. 1.9 Bac. 6.3		Bacitracin Erythromycin	Expired
44 F	Lupus erythematosus Cortisone and ACTH	Pen. >15.6 Tetra >31.2	Chlor. 7.8 Eryth. 3.9 Bac. 3.2		Oxytetracycline Erythromycin	Expired Autopsy
54 M	Lymphatic leukemia Metacorten therapy	Pen. >15.6 Tetra. >15.6	Chlor. 7.8 Eryth. 1.0 Bac. 1.6		Chlortetracycline Erythromycin Streptonivicin Metacorten	Expired Autopsy
26 F	Rheumatic heart disease Cortisone				Penicillin Streptomycin Tetracycline Erythromycin Cortisone	Expired Autopsy
16 F	Lupus erythematosus				Penicillin Streptomycin Cortisone ACTH	Expired Autopsy
72 M	? Rheumatic heart disease Pyoderma				Penicillin	Expired Autopsy
54 M	Rheumatic heart disease				Penicillin Streptomycin	Expired Autopsy
39 M	Incision of perianal abscess Reticuloendotheliosis				Chlortetracycline Penicillin	Expired Autopsy
76 M	Catheterization of urinary bladder Pyelonephritis				Penicillin Streptomycin	Expired Autopsy
53 M	Severe body burns Thrombophlebitis				Penicillin Streptomycin Tetracycline	Expired Autopsy

Pen., penicillin; strep., streptomycin; tetra., tetracycline; c-tet., chlortetracycline; o-tet., oxytetracycline; chlor., chloramphenicol, eryth., erythromycin, bac., bacitracin

Table 5
Causative Factors in 41 Cases of Staphylococcal Septicemia

1. Number of Cases Having Infections Acquired in the Hospital		24
A. Postoperative wound infections	13	
B. Following minor surgical procedures	8	
C. Following cortisone therapy for chronic disease	2	
D. Neonatal infection	1	
2. Number of Infections Acquired Outside of the Hospital		17
A. Portal of infection known	8	
B. Portal of infection not known	7	
C. Following cortisone therapy for chronic disease	2	

come of the illness and the sensitivity of the organisms to penicillin. There were 16 hospital cases of septicemia whose infections were caused by strains that resisted penicillin in a concentration of 15.6 units per ml. or more, and 14 of these patients died. Although comparative data with nonhospital infections are insufficient, it is of interest that, of 4 patients having infections due to sensitive strains, only 1 died, and death was due to cardiac failure.

Epidemiological Investigations of Wound Infections Due to Penicillin-Resistant Staphylococci

While there is ample evidence that the reservoir of antibiotic-resistant staphylococci in a general hospital resides on the mucous membranes and skin of hospital personnel and in the immediate environment of the hospital, precise details are lacking concerning the spread of staphylococci to patients. In order to get more definite information concerning the carrier rate of resistant staphylococci at the University Hospitals, an extensive epidemiological investigation was undertaken in 1954.[5] Since the problem at hand appeared to center on postoperative wound infections, attention was directed to the personnel on the surgical staff, including the surgeons, nurses, and ward attendants.

Coagulase-positive staphylococci were isolated from the nasopharynx of 32 per cent of 208 members in the surgical group. The striking feature was that 41 per cent of those with positive cultures were harboring staphylococci that resisted more than 1,000 units of penicillin per ml. This was in contrast to the control information obtained on an equivalent number of patients seen in the outpatient department. The majority of these individuals coming from rural areas were carrying penicillin-sensitive strains of staphylococci. Data were also obtained from patients in the hospital having staphylococcal infections, and the results paralleled those found in the surgical personnel. A summary of the results acquired in the 3 groups is given in TABLE 6. These data support the conclusions of others that the members of a hospital staff and infected patients harbor antibiotic-resistant staphylococci to a considerable degree.

While the foregoing data suggest a causal relationship between healthy carriers of resistant staphylococci and the infections appearing in patients, more definite proof is desired, and this was afforded by a study carried out in cooperation with the Minnesota Department of Health in 1954. A small newly-constructed hospital serving a community near Minneapolis was first opened in 1953. The first patient operated upon suffered from a postoperative wound

TABLE 6

Group	Units of penicillin per ml.					
	0.1–1.0	1.0–10	10–100	100–500	500–1000	>1000
Surgical staff (208)	19.1	2.9	29.4	7.3		41.1
Inpatients (66)	5.2	3.1	8.3	33.3	9.3	40.6
Outpatients (200)	60.9	7.3	21.9	9.7		

Comparative resistance of strains of *M. pyogenes* to penicillin (units per ml.) isolated from 3 different groups at the University of Minnesota Hospitals in 1954. The results are expressed in per cent of strains isolated. The total number studied in each group is given in parentheses.

infection caused by penicillin-resistant staphylococci. In succession, a total of 10 patients with postoperative wound infections were brought under observation. Since all of the infections were due to coagulase-positive staphylococci highly resistant to penicillin, an appeal was made to the Minnesota Department of Health to try and elicit the source of these infections. An agar plate exposed for 90 minutes near the site where surgical instruments were rinsed showed the presence of 65 colonies of hemolytic staphylococci. On another occasion when no surgery was in progress, a single colony of *Staphylococcus* appeared on the plate after prolonged exposure. Cultures of the nasopharynx were carried out on the attending staff, and a penicillin-resistant strain of *Staphylococcus* was isolated from the scrub nurse who had been in attendance during all of the operations. This strain, plus the strains isolated from 3 patients, were submitted to Robert Wise for bacteriophage typing. Lysis of the staphylococcal cultures resulted from a group of phages, leaving no doubt that the scrub nurse was the source of the infections. The sequence of events and the results of bacteriophage typing in this group of infections are shown in FIGURE 1. No further infections occurred following the removal of the scrub nurse from the operating room.

Staphylococcal Enterocolitis

A disturbing side effect of antibiotic therapy is the appearance of diarrhea, which may be associated with pseudomembranous enterocolitis and antibiotic-resistant staphylococci. We have encountered only 1 fatality in which the appearance of a severe enterocolitis and resistant staphylococci was the primary cause of death. Such a staphylococcal enterocolitis, however, has been a contributing factor in the death of several other patients. This complication of antibiotic therapy is usually associated with the tetracycline drugs, but we have seen it result from all of the commonly used antibiotics when administered parenterally as well as orally. A severe membranous enterocolitis was observed in a young adult who had been given penicillin and streptomycin prophylactically following surgery. A pure culture of *Staphylococcus* resistant to these 2 antibiotics was recovered in the purulent stools. We encounter 1 to 2 patients a month at the University Hospitals who have severe diarrhea associated with antibiotic-resistant staphylococci in the stools. Perhaps we have seen less of this complication than has been seen at other hospitals because the

EPIDEMIOLOGICAL DATA ON SURGICAL WOUND INFECTIONS DUE TO PENICILLIN-RESISTANT STAPHYLOCOCCI WITH BACTERIOPHAGE TYPING

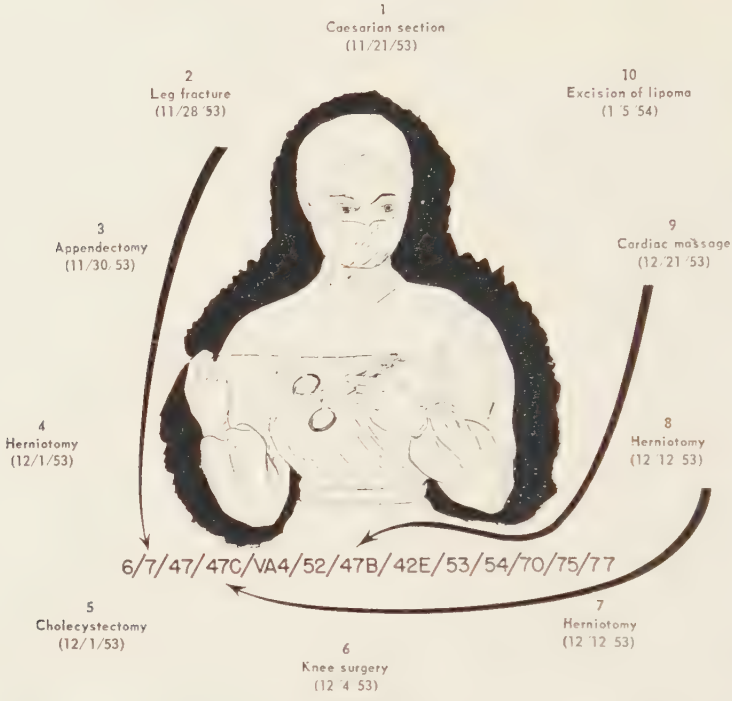

FIGURE 1

majority of our surgeons have been adverse to the routine use of the tetracycline drugs in the preparation of patients for bowel surgery. When an antibiotic has been used for this purpose, neomycin is most commonly selected. Neomycin-resistant staphylococci have not been a problem with us, although the antibiotic is quite toxic when administered parenterally.

What Can Be Done About the Control of Hospital Infections Due to Antibiotic-Resistant Staphylococci?

Since a major concern of hospital infections is related to wound infections, both surgically induced and traumatic, every attempt should be made to enforce strict aseptic techniques in the operating rooms and to keep the immediate environment meticulously clean. These aspects of the problem have been admirably explored by Blowers and his associates.[7] Patients with staphylococcal sepsis are often hospitalized on services other than the surgical. The control of cross infections in a hospital can be further accomplished by the enforcement of a uniform rule stating that all patients having staphylococcal sepsis should be strictly isolated. This regulation applies particularly to patients having open and exuding wounds and to those with staphylococcal respir-

atory infections. The immediate environment of these patients, containing such items as bed clothing, bed pans, and dust, is often heavily contaminated with staphylococci. Finally, there is an urgent need for a drug that is not only highly active against staphylococci but relatively nontoxic for the patient. Most important, however, the potential appearance of resistant organisms under the influence of such a drug should be reduced to a minimum. The availability of such a drug might once again reduce the terrifying mortality rate that confronts the clinician today in the management of staphylococcal septicemia.

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Discussion of the Paper

Chester W. Howe (*Boston University School of Medicine, Massachusetts Memorial Hospitals, Boston, Mass.*): In the field of postoperative wound infections following clean surgery, I agree with Wesley W. Spink that much can be done to control staphylococcal sepsis by more strict attention to the prevention of cross contamination. This is one of the specialized situations in hospitals, referred to earlier by Walsh McDermott, in which we are seeing a high incidence of infections due to *Staphylococcus aureus* occurring sporadically or in small epidemics, and apparently related to a high carrier rate of antibiotic-resistant organisms in hospital patients and personnel. My contacts with hospitals and surgeons in the Boston area and elsewhere suggest that the problem is more frequent and more serious than is indicated by the few reports in the literature.

A brief review of our experience in the Department of Surgery at the Massachusetts Memorial Hospitals will serve to illustrate the problem.[1] In 1949 our wound-sepsis rate for major and trivial infections combined, following clean operations, was 1 per cent. This rate gradually increased over a five-year period in a stepwise, statistically significant fashion so that in 1953 it had reached 4 per cent, with a peak of 10 per cent for a period of 1 month early in 1954. During these years, so-called prophylactic antibiotic therapy with penicillin was widely used before and after surgery. A survey of patients and personnel in the summer of 1953 showed that 99 per cent of us were nasopharyngeal

or skin carriers of *Staphylococcus aureus*, and that 75 per cent of the strains were penicillin-resistant. Eighty per cent of our wound infections in 1953 were due to this organism.

We started a program consisting of various techniques to reduce cross-contamination. Without going into all the factors bearing upon the development of wound infection, I might mention a few of the measures taken. The use of routine antibiotic prophylaxis was largely curtailed. Since the majority of our infections were deep seated and their pathology indicated that the wounds were seeded in the operating room, and since the nasopharynx is the main reservoir for staphylococci, we advocated double masking and the changing of masks every hour or 2 during long operations. On the wards, we advocated the use of masks and gloves by doctors and nurses, while changing septic dressings, in order to help prevent themselves from becoming heavy carriers. Special "septic sets" were provided for dressing infected wounds. All instruments were immediately wrapped following dressings and sent to be autoclaved before cleaning. They were then definitively sterilized before being used again. Waxed-paper bags were provided for the disposal of contaminated dressings. Isolation of patients with infected wounds proved to be impractical in our hospital, but isolation of the wounds by the use of occlusive dressings and isolation of the nasopharnyx by the use of masks are feasible. Early control of serious wound infections by drainage, debridement, and surgical closure was carried out whenever possible.

Following the institution of these and other measures, our infection rate was cut in half during the next 2 years so that in 1955 it was down to 2 per cent. Moreover, only 56 per cent of these infections were due to *Staphylococcus aureus*, in contrast to 80 per cent in 1953. A repeat survey of the patient and personnel carrier rate last summer showed that it had been reduced from 99 to 75 per cent, 62 per cent of the strains being penicillin resistant. Our statistician tells me that these reductions are quite significant.

Discussions with surgeons from various other hospitals strongly suggest that *Staphylococcus aureus* wound-infection rates, seldom published, have been much higher than those presented here. Blowers *et al.*[2] reported from England that postoperative wound infections with penicillin-resistant *Staphylococcus aureus* became so frequent in 1952 (10.9 per cent) that it was necessary to close the hospital. Following extensive reorganization, the rate fell to 3.9 per cent in 1953 but rose to 6.2 per cent in 1954. In the past it has been considered that on a well-regulated surgical service the wound-infection rate should not exceed 1 or 2 per cent. Although we are again approaching that level, we feel that further improvement is possible, and we are carrying out studies directed toward that end, especially on the house service, where the high average age and poor-risk character of the patients noted in recent years makes this problem especially important.

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Part IV. Clinical Staphylococcal Infections

STAPHYLOCOCCAL INFECTIONS CURRENTLY ENCOUNTERED IN A LARGE MUNICIPAL HOSPITAL: SOME PROBLEMS IN EVALUATING ANTIMICROBIAL THERAPY IN SUCH INFECTIONS*

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The topic originally designated for this paper, "The Effect of Present Antimicrobials in Staphylococcal Infections" was presumably chosen in order to present in this monograph a clear picture of the status of antimicrobial therapy as applied to the kinds of staphylococcal infections now being encountered. This topic proved to be rather difficult, and neither profitable nor even capable of clear definition, either from our own experience or from data readily available in the literature. Much of the difficulty seemed to stem from the lack of any large body of basic data on the occurrence of various types of staphylococcal infections. Because 1 important objective of this monograph was interpreted to be the definition of some of the major problems involved in staphylococcal infections and an exploration into possible methods of coping with them, it seemed proper to deviate somewhat from the assigned topic in order to present some data and observations arising out of an attempt to evaluate antibiotic therapy. These will concern 2 general topics: first, the magnitude of the problem of staphylococcal infections as encountered at the Boston City Hospital in recent months and, second, some problems involved in evaluating the effectiveness of antibiotics in the management of severe staphylococcal infections.

Spot Survey, January 1956

In preparation for this paper, a spot survey was made of all of the wards of the Boston City Hospital, Boston, Mass., during the latter part of January 1956. Accompanied by the Resident and other members of the house staff of each ward, along with the supervising nurse and, in some instances, by the Director of the service, we passed from bed to bed, noting primarily 3 points with respect to each patient: first, whether there was a staphylococcal infection of any type, second, whether it was present at the time of admission to the hospital or acquired (or first recognized) after admission and, third, whether any antimicrobial therapy had been given immediately prior to or after its clinical appearance or recognition.

Although the third aspect would seem to be the most important one for the present purpose, the findings on this phase were quite unrevealing. Very few of the patients whose staphylococcal infections were present on admission to

* Aided by a grant from the National Institutes of Health, Public Health Service, Department of Health, Education, and Welfare, Bethesda, Md.

the hospital had previously received antimicrobial therapy, whereas such treatment had been used in about one half of those whose staphylococcal infections were acquired in the hospital. Penicillin plus streptomycin had generally been used either in "prophylaxis" or when infection was suspected and its nature was not clearly defined. These and other antibiotics had been used when indicated for other infections that were present before the staphylococcal infection was recognized. The findings on the first 2 aspects are of greater interest and will be presented in more detail.

A summary of these findings is shown in TABLE 1. It will be seen that the total number of patients in the hospital at the time of the survey was approximately 1172. This included patients on all services: medical, surgical, pediatric, and the specialties. A total of 181 patients was found to have some type of manifest staphylococcal infections. Of these, 68 had the infections at the time of admission to the hospital. These cases were arbitrarily divided into serious and less serious infections, although some of the latter infections were quite serious from the point of view of prolonged morbidity and were potentially even more serious than the others, occurring as they did in patients with diabetes, impaired circulation, cardiac or renal failure, or other severe chronic or acute diseases.

Infections acquired outside of the hospital. Among the 15 serious infections are included: 6 cases of osteomyelitis; 3 of pneumonia and empyema; 3 with severe sinusitis and facial cellulitis; 2 cases of bacterial endocarditis, including one that also had osteomyelitis; 1 of peritonitis; and 1 admitted with a carbuncle and also having bacteremia and metastatic lesions. Among the 53 less-serious infections present on admission, there were furuncles, felons, or carbuncles in 20; infections of burns, rashes, ulcers (including decubitus ulcers),

TABLE 1

SPOT SURVEY OF STAPHYLOCOCCAL INFECTIONS

Boston City Hospital, January 1956

Number of patients surveyed	1172

1. *Infections present at time of admission (68)*

Serious infections (15)		*Less-serious infections (53)*	
Osteomyelitis	6	Infected burns, rashes, ulcers, and wounds	23
Sinusitis and cellulitis	3	Furuncles, felons, carbuncles	20
Endocarditis	2	Breast abscesses	6
Pneumonia and/or empyema	2	Chronic suppurative otitis	2
Carbuncle with bacteremia	1	Urinary tract infection	2
Peritonitis	1		

2. *Infections probably acquired in the hospital (113)*

Serious infections (16)		*Less-serious infections (97)*	
Pneumonia and empyema	6	Burns, wounds, ulcers	52
Burns, wounds, ulcers	3	Furuncles, abscesses	39
Bacteremia, ? source	2	Respiratory tract	5
Carbuncles, soft-tissue infect	2	Urinary tract	1
Meningitis, postop	1		
Subphrenic abscess, postop	1		
Infected "cutdown" + bacteremia	1		

and wounds acquired outside of the hospital totaled 23; 6 patients with breast abscesses all of whom had given birth at this hospital and had been discharged with their babies only to be readmitted within 3 days to 3 weeks; 2 had staphylococcal infections of the urinary tract; and 2 had acute and chronic otitis media. Some of these cases, particularly those with superficial infections of wounds, burns, or ulcers were mixed infections in which other organisms also participated. Thus, 38 per cent of the staphylococcal infections noted at the time of the survey had been acquired outside of the hospital.

The extent of the current problem of staphylococcal infections outside of the hospital, that is, in the community from which the patients are derived, is not entirely clear, but it is known to be quite extensive from the large number of patients being treated in the Outpatient Department, by physicians of local domiciliary medical services, and by district nurses. Exact figures from these sources, however, were not compiled.

Infections acquired in the hospital. The rest of the staphylococcal infections observed, numbering 113, or 62 per cent of the 181 staphylococcal infections, were apparently acquired after admission of the patients to the hospital. Sixteen of these were listed as serious and the others as less serious, but many of the latter were causes of severe and prolonged morbidity. The 16 serious infections included: 6 patients with pneumonia and empyema, originally admitted to the hospital for other systemic diseases or for respiratory infections due to other organisms; 3 with severe secondary infections of wounds and burns; 1 with a posteraniotomy meningitis; 1 with an infected venous "cut-down" associated with prolonged bacteremia; 2 with extensive carbuncles and infections of soft tissue; 1 with a subdiaphragmatic abscess occurring after a clean laparotomy; and 2 were cases of staphylococcal bacteremia, the source of which had not yet been determined. The 97 patients with infections classified as less severe included: 52 with secondary infections of primarily clean surgical wounds as well as of burns and ulcerations, particularly decubitus ulcers acquired after admission to the hospital in severely ill patients or in patients who had had severe injuries, fractures, *etc.*; 39 with multiple furuncles or abscesses of various sizes that yielded staphylococci; 5 with pulmonary infections in which the *Staphylococcus* seemed to be the primary organism concerned at the time of the survey but was not present at the time of admission; and 1 was a urinary-tract infection acquired following instrumentation.

Infections in hospital personnel. The nosocomial infections shown in TABLE 1 did not include cases among the resident house staff, nurses, or service personnel of the hospital. Among the house staff there were 18 with large furuncles or carbuncles, some of which involved only unexposed parts. Interestingly enough, these members of the house staff were widely distributed among the various services: 6 of them were on the general surgical services; 7 on medical services; 2 were orthopedic surgeons; 2 were pathologists and 1 was an anesthesiologist. There were at least 9 others of the resident staff who had recently recovered from staphylococcal infections. Cursory inquiry into the situation among nurses revealed that 7 of them were absent from duty because of staphylococcal infections, of whom 5 were hospitalized at the time for these

infections, and 9 others had recently been treated for staphylococcal skin infections. There were also at least 8 ward attendants with significant infection of the skin of exposed surfaces that were known to the supervising nurses. These attendants had either just returned to duty or were still out sick with the infection at the time of the survey. The house surgeons and physicians, incidentally, in most instances had been hospitalized at least part of the time for these infections, and the surgeons were excluded from operating for prolonged periods because of the involvement of the skin of the forearm and hands. There undoubtedly were more doctors and nurses with staphylococcal infections that were not obvious and that were not uncovered by the superficial type of survey that was made.

Susceptibility of strains to antibiotics. Neither this spot survey nor the other clinical material to be presented includes detailed bacteriologic studies of the strains of staphylococci concerned in the infections or of their susceptibility to the currently available antibiotics, another feature that was presumably implied in the subject originally assigned for this paper. Considerable experience with such studies during the last few years and actual tests of a large proportion of the strains from cases included in the present studies make it reasonably certain, however, that the current picture at the Boston City Hospital is similar in this respect to that being reported from many other large hospitals throughout this and other countries. The staphylococci from the majority of "closed" infections present at the time of admission to the hospital in patients not recently exposed to the hospital environment have proved to be susceptible to almost all of the available antibiotics except polymyxin. Staphylococci from infections acquired in the hospital, on the other hand, have quite uniformly proved to be resistant to penicillin and the tetracyclines, a large proportion being found resistant to streptomycin and a small proportion are now proving to be resistant to erythromycin. Nearly all, however, are at least moderately susceptible to chloramphenicol, erythromycin, bacitracin, and neomycin, and many to streptomycin as well. It is for that reason that treatment of the nosocomial infections has, in recent years, been largely with combinations of the latter antibiotics.

Staphylococcal Infections in Autopsied Cases

Another aspect of the current or recent status bearing on the magnitude of the problem of staphylococcal infections at this hospital was investigated through a study of all the autopsies that were done at the Mallory Institute of Pathology, of this hospital, during the year 1955.* In almost all autopsies, except those done long periods after death and some others, cultures were made with accepted bacteriologic techniques. Cultures were obtained, more or less routinely, from cardiac blood, spleen, and each lung and, in addition, cultures of other foci, especially purulent areas, were also obtained when these were encountered under conditions that excluded gross contamination. The observations are, of course, subject to various technical and other criticism but, since this consideration is not crucial with respect to the main purpose of the present survey, technical aspects will not be discussed in detail.

* We are indebted to G. Kenneth Mallory and Stanley L. Robbins for permission to use these data from their protocols and also to the resident pathologists and assistant pathologists who conducted the routine autopsies and made the cultures upon which this survey is based.

TABLE 2

HEMOLYTIC *STAPHYLOCOCCUS AUREUS* IN POST-MORTEM CULTURES
Boston City Hospital (Mallory Institute of Pathology), 1955

Total number of autopsies performed		1039
Number in which cultures were done		914*
Hemolytic *Staphylococcus aureus*, pure or predominant		266
Cardiac blood and spleen, both positive	35	
Cardiac blood positive, spleen negative	32	
Spleen positive, cardiac blood negative	19	
Total: cardiac blood and/or spleen positive		86
Other significant sites positive (cultures of cardiac blood and/or spleen either negative, not done or contaminated)		
Peritoneal exudate, free or localized	19	
Pericardial exudate	6	
Joints, bones, mastoids	5†	
Cardiac valves (vegetations)	4	
Pleural exudate	4	
Renal abscess or pyonephrotic exudate	4	
Lung abscess (2), mediastinal abscess (1)	3	
Brain and meningeal exudate	2	
Total: other significant sities		47
Cultures positive for both hemolytic and nonhemolytic *Staphylococcus aureus*		
Spleen positive, cardiac blood negative	3	
Cardiac blood and spleen, both positive	2	
Cultures positive for nonhemolytic *Staphylococcus aureus*		
Spleen positive	8‡	
Cardiac blood positive	2	

* Those in which cultures were not made included mostly stillborn and newborn infants, unclaimed bodies in which autopsies were performed 5 days or longer after death, and 5 cases from the Sanatorium (tuberculosis) Division in which only stained smears were made.

† One also had a moderate number of nonhemolytic *Staphylococcus aureus*.

‡ Hemolytic *Staphylococcus aureus* was grown in pure culture from cardiac blood in 4 of these cases.

Some of the significant bacteriological findings in this autopsy material as they relate to staphylococcal infections are summarized in TABLE 2. The total number of autopsies performed during the calendar year 1955 was 1039, and satisfactory cultures were made in 914 of these cases. The findings with respect to staphylococci are reported primarily on the basis of the morphological appearance of the colonies, that is, their color and hemolytic activity on sheep's-blood agar. For purposes of this presentation, those organisms that failed to produce yellow pigment, that is, those classified as *Staphylococcus albus*, were excluded. A total of 266 cases was found in which hemolytic *Staphylococcus aureus* was cultivated as the only or predominant organism from the heart's blood, spleen, lungs, or various important primary foci of infection.

Post-mortem evidence of invasion of the blood stream. Among these 266 cases, 86 yielded hemolytic *Staphylococcus aureus** from the heart's blood, spleen, or both: 32 from heart's blood alone, 19 from spleen alone, and 35 from both

* The descriptive terms "*Staphylococcus aureus*," for those failing to show hemolysis, "hemolytic *Staphylococcus aureus*" when hemolysis was present, and "*Staphylococcus albus*" for those without yellow pigment and without hemolysis are used here because they correspond to the manner in which these strains were recognized and classified. While coagulase tests were done on only a relatively small number of the strains, those of hemolytic *Staphylococcus aureus* usually were coagulase positive and were found to be so in the strains studied and tested in our laboratory. Strains of *Staphylococcus albus* were invariably negative by the coagulase test, and those classified as nonhemolytic *Staphylococcus aureus* sometimes produced hemolysis when examined on horse blood agar, and some of them did produce coagulase.

heart's blood and spleen. The conditions under which these cultures were obtained have led the pathologists and bacteriologists to feel that the cultures of the spleen are more significant or, rather, less likely to be contaminated than those from the heart's blood, and this would account for many cases with positive heart's-blood cultures that had to be excluded because of gross contamination. In addition to these 86 cases, there were pure cultures of nonhemolytic *Staphylococcus aureus* found in the spleens of 8 patients, and in 2 cases this was cultured from heart's blood. Interestingly enough, of the 8 patients from whom pure cultures of nonhemolytic *Staphylococcus aureus* were obtained from the spleen, 4 had hemolytic *Staphylococcus aureus* in pure culture in the heart's blood. There were 5 additional patients in whom both hemolytic *Staphylococcus aureus* and nonhemolytic *Staphylococcus aureus* were cultured, 3 of them in the spleen alone and 2 in both heart's blood and spleen. These data are of interest in relation to a parallel survey of staphylococcal bacteremias that will be mentioned later.

Focal staphylococcal infections. In addition to these evidences of bacteremia obtained post mortem, there were 47 patients among the 266 from whom hemolytic *Staphylococcus aureus* was obtained either in pure culture or as the predominant organism from significant foci, but this organism was not obtained either from the heart's blood, spleen, or lungs, that is, where the only source of hemolytic *Staphylococcus aureus* was an important focus of infection and cultures from other sources either were not done, did not yield this organism, or were grossly contaminated. These included: 4 cases of vegetative endocarditis with hemolytic *Staphylococcus aureus* cultured directly from the valve; 6 with positive cultures of pericardial exudate; 4 of pleural exudate; 19 of peritoneal exudate found either free or in localized pockets or abscess cavities; 5 of infected joints, mastoids or other bones; 2 of lung abscess; 1 of mediastinal abscess; 4 of renal abscesses or pyonephrotic exudate; and 2 of meningeal exudate. There was 1 additional case, not listed in the table, in which a nonhemolytic *Staphylococcus aureus* was found in the brain and another in which such an organism was cultured from a suppurating mastoid, both occurring in pure culture.

Pathologic significance of post-mortem bacteriological findings. A brief review was made of the autopsy protocols, attention being given to the correlation between the final anatomical diagnosis and the bacteriological findings at autopsy. This indicated only that without more detailed study the relationship of these bacteriological findings to the anatomical lesions could not be interpreted in many instances. That was particularly true of the patients with staphylococci other than hemolytic *Staphylococcus aureus*, but it was also true of some of the patients in whom such strains were obtained in relatively small numbers, or together with other species that seemed to be more pertinent, or where hemolytic *Staphylococcus aureus* was obtained in the apparent absence of any recognizable suppurative lesions. A study of a number of clinical records of cases in which nonhemolytic *Staphylococcus aureus*, and even some in which *Staphylococcus albus*, was cultured during life, however, indicated that these may be associated with significant lesions. There were several cases, for example, in which nonhemolytic *Staphylococcus aureus* or *Staphylococcus albus*

was obtained from blood cultures on 1 or more occasions during life, in which foci of suppuration yielding pure cultures of hemolytic *Staphylococcus aureus* were demonstrated clinically or noted at post mortem examination. Not shown in TABLE 2 and not included among the 266 cases analyzed were 198 additional cases with either nonhemolytic *Staphylococcus aureus* or *Staphylococcus albus* predominating in autopsy cultures. Many of these also had hemolytic *Staphylococcus aureus* in small numbers from some sources, either alone or mixed with the other staphylococci, but not predominant or in pure culture in any organ.

Staphylococcal Bacteremia

Another aspect that was studied in arriving at some estimate of the magnitude of the problem of staphylococcal infection was concerned with cases of bacteremia demonstrated during life.* For this purpose, the records of all patients from whom blood cultures were obtained that yielded hemolytic *Staphylococcus aureus* were reviewed in an attempt to determine the relationship of the positive blood culture to the disease in the patient and, where possible, to evaluate the therapy that was used. In this portion of the study, which is still in progress, we encountered major difficulties.

Clinical significance of staphylococcal bacteremia. In the first place, regardless of the bacteriological criteria used, which here, too, included primarily morphological characteristics of the colonies when transplanted to blood-agar plates and tests for coagulase production in many instances, we encountered apparently pathogenic strains of staphylococci that, when correlated with the clinical findings, must be considered either as transients or as contaminants in the blood stream. It was by no means a simple task to determine whether an organism fell into one of these categories or was actually a cause of the patient's disease. At this hospital, as elsewhere, blood cultures are drawn primarily from patients with febrile illnesses and, most often, from patients with symptoms that are indicative of infections that may be serious. The known high carrier rate and prevalence of pathogenic staphylococci in the nose, throat, and skin of hospital personnel and of patients, as well as the large numbers of the same organisms found in the environment in many large hospitals (and presumably also in this hospital, although this condition was not studied specifically) offer ample opportunities for contamination with such pathogenic strains.

Variety of staphylococci found in blood cultures. Nevertheless, it is of interest to note these very preliminary figures for cases in which various kinds of staphylococci were obtained from blood cultures. These are shown in TABLE 3. Here, too, the classification is based primarily on pigment production and hemolysis on blood-agar plates. The isolation and identification was carried out in the hospital bacteriology laboratory. Many of these strains, however, have been studied or stored for future study in our laboratory, but the data on them are not included in this analysis. The patients classified as showing *Staphylococcus aureus* had such strains, with or without hemolysis, in 1 or more blood cultures,

* We are indebted to Marion E. Lamb, A. Kathleen Daly, and their associates in the Bacteriological Laboratory of the Mallory Institute of Pathology who performed the bacteriological examinations.

Table 3

Patients with Bacteremia at Boston City Hospital, 1955

Staphylococcal Bacteremia

Variety*	Number	Died	% Died
aureus, with or without hemolysis	196	89	45
" hemolytic	106	38	36
" nonhemolytic	90	51	57
albus	384	70	18

Other Bacteremias

Pneumococcus (typed)	78	*Escherichia coli*	67
Streptococcus viridans	47?	*Proteus* species	64
Enterococcus	21	*Aerobacter aerogenes*	54
Meningococcus	11	*Bacillus fecalis alcaligenes*	19?
Hemophilus influenzae	6	*Pseudomonas aeruginosa*	13
Streptococcus pyogenes	4	*Klebsiella pneumoniae*	3
Microaerophilic *Streptococcus*	3	*Salmonella*	1
Gonococcus	2	*Bacteroides*	1

* The numbers, particularly those on mortality, are preliminary and uncorrected. All patients with blood cultures positive for the organisms listed are included, and some patients are included under more than one organism. ? denotes that many of these cultures were thought to be contaminants (often mixed with other species). There were, in addition, 11 patients, not listed, whose blood yielded *Candida*, and 364 from whose blood only organisms considered to be contaminants were grown (these include: diphtheroids, *Bacillus subtilis*, unidentified chromogenic gram-negative bacilli, *Micrococcus tetragenus*, *Micrococcus citreus*, and *Neisseria flavus*). Cultures of blood taken post mortem are not included in this table.

but may also have had *Staphylococcus albus*. On the other hand, cases listed as showing *Staphylococcus albus* had blood cultures yielding only this organism on 1 or more occasions. The data on mortality are preliminary and uncorrected.

It is seen that 18 per cent of the 384 patients from whose blood cultures *Staphylococcus albus* was isolated ended fatally. While these figures may bear little if any relation to the problem of staphylococcal infections, except perhaps as they add to difficulties in interpretation, there is little doubt that some of these organisms were significant in an occasional case, as for example where there was either a definite diagnosis made of bacterial endocarditis or where there was a high likelihood that that disease was present. Patients with *Staphylococcus aureus* in 1 or more blood cultures numbered 196 and, of these, 89 died, a mortality of 45 per cent. Some of these patients had *Staphylococcus albus* in the same or different cultures.

Some of the difficulty in interpreting these figures is seen from the further breakdown of the cases having *Staphylococcus aureus*. This tabulation indicates that among the 106 patients whose blood cultures at any time yielded hemolytic *Staphylococcus aureus* there were 38 deaths, or 36 per cent, whereas among 90 from whom only nonhemolytic *Staphylococcus aureus* was obtained from blood cultures during life there were 51 deaths, a mortality of 5 per cent. To be sure, in many of the latter cases where autopsies were performed, *Staphylococcus aureus* showing definite hemolysis was isolated, often in pure culture, from materials obtained post mortem. The significance of these figures is by no means clear.

Bacteremia due to organisms other than staphylococci. The numbers of patients in whom bacteria of various other genera were obtained from blood cultures during life are also shown in TABLE 3. They are of interest here mainly because they attest to the primary position of the *Staphylococcus* among bacteria causing invasive infections at the present time. Such data, particularly when studied in greater detail and in relation to similar material obtained at different times and under comparable circumstances, may shed some light on some of the over-all effects of the general use of antimicrobial therapy.

Varieties of staphylococci occurring in same patient. In regard to the varieties of staphylococci it is also worth noting that both hemolytic and nonhemolytic colonies of *Staphylococcus aureus* were obtained on a number of occasions from 1 and the same culture in many instances, and sometimes from different cultures in the same patient. This was well illustrated in a patient with prolonged bacteremia whose blood cultures yielded staphylococci repeatedly over a period of 4 months and were negative on only 4 occasions. Some of these cultures yielded only hemolytic *Staphylococcus aureus*, others only nonhemolytic *Staphylococcus aureus*, and still others, both types from the same culture. The only other organisms grown from the same series of blood cultures were "diphtheroids." These occurred alone on 3 occasions and together with staphylococci in 7. Interestingly enough, *Staphylococcus albus* was never grown from any of these blood cultures, numbering 70 in all. *Staphylococcus albus*, however, was cultured from the original wound that was presumably responsible for the infection. The only other cultures made from the exudate of this wound yielded a nonhemolytic *Staphylococcus aureus*. A diphtheroid was also grown from the same wound cultures on both occasions. The last 5 cultures before death yielded only hemolytic *Staphylococcus aureus*. At autopsy, blood obtained from the right ventricle and from the inferior vena cava, as well as cultures of the tricuspid valve and of the left lung, all yielded hemolytic *Staphylococcus aureus*. The spleen, on the other hand, yielded nonhemolytic *Staphylococcus aureus* in pure culture, and the right lung yielded a moderate number of staphylococcal colonies, some of which were hemolytic and others nonhemolytic.

While it has been tempting to ascribe changes in morphology and growth characteristics to acquisition of resistance or to other effects of the therapeutic agent used, the possibility of successive or concurrent invasions by strains of different susceptibility, as permitted by the therapy employed, has also to be considered. This is also illustrated in the following case from an earlier survey of antibiotic resistance of staphylococci at this hospital.[1] The data shown in TABLE 4 indicate that at intervals of 4 days the organisms cultured from the blood changed in their susceptibility to penicillin and oxytetracycline. The first culture before treatment was started was sensitive to both of these antibiotics, but 4 days after the administration of penicillin the strain was resistant to penicillin but still sensitive to oxytetracycline. Four days after that, the patient having received oxytetracycline in the interim, the organisms isolated from an abscess and from the blood were highly resistant to both penicillin and oxytetracycline. As might be expected, the original strain did not produce penicillinase, but the other 3 strains did. It would have been tempting to as-

TABLE 4
ANTIBIOTIC SUSCEPTIBILITY OF STRAINS OF HEMOLYTIC *STAPHYLOCOCCUS AUREUS* IN ONE PATIENT

Date*	Source	Previous therapy†	M.I.C.‡	
			P	OT
16 Jan.	blood	O	0.04	4
20 Jan.	blood	P	>400	4
24 Jan.	abscess	P, OT	>400	>400
24 Jan.	blood	P, OT	>400	>400

* 1952.
† O = none, P = penicillin, OT = oxytetracycline.
‡ M.I.C.—minimum inhibiting concentration, μg. per ml.

sume that in this case the organisms acquired resistance in the body. This patient had a repair of an arteriovenous aneurysm, however, and an infection occurred at the site of the repair. Unfortunately, a culture of the abscess that developed was not obtained until the time of the last of these blood cultures and, at that time, it yielded organisms resistant to both of the antibiotics that had been used. It is presumed that the original infection of the blood stream was with a sensitive strain and that resistant strains prevalent in the patient or his environment reinfected or simultaneously infected the wound. The original sensitive organisms were eliminated by treatment, and the organism finally cultured from the blood was resistant to both of the antibiotics that had been used.

Evaluation of Chemotherapy

The exact significance of the matters discussed thus far to the problem of the efficacy of the currently available antibiotics is by no means clear, but these drugs certainly have some important bearing. In the first place, the findings in a large proportion of the patients in whom staphylococcal infections were demonstrated and bacteriologically confirmed at autopsy, and even in those in whom no clear-cut or advanced staphylococcal infections were observed but from whom staphylococci of apparently pathogenic strains were cultured from the vital organs, indicate that staphylococcal infection may have been responsible for, or at least contributed to, the fatal outcome. In some, the conditions suggest terminal, if not preterminal, invasion, and do not necessarily give a true indication of the effect of antibiotics. To be sure, many of these patients had received one or more antibiotics, either simultaneously or in succession. Of these, a respectable number were treated according to the results of tests of sensitivity of the strains available ante mortem. As already mentioned, however, the bacteriologic data do not give clear or decisive indications as to the amount of pathology ascribable to the *Staphylococcus;* nor can it be said that all, or even the majority, of these patients died under conditions in which the *Staphylococcus* could be considered as the cause of death, although the probability that it contributed to the fatal outcome must be entertained. Undoubtedly, also, some of these patients had an insufficient period of treatment to indicate that these were treatment failures. The majority of cases, however,

were serious and fatal diseases in the aged and, in many of them, the staphylococcal infection was not suspected and therefore was not treated. In other cases, treatment had been given empirically because of the occurrence of fever and, in many of these patients, staphylococci or other organisms were cultured from various sites and treatment given accordingly.

It was thought that a survey of the data in patients from whom positive blood cultures were obtained during life would be more revealing since it could be considered that such patients had serious staphylococcal infections. A preliminary review of the data in these cases, however, including many where there was an opportunity to follow the course of the illness rather closely, indicates that the evaluation of the effects of antibiotic therapy is extremely difficult.

The nature of the staphylococcal infections is such that the efficacy of treatment in mild cases of simple superficial infection or even of deeper and more-extensive infections such as carbuncles is difficult to evaluate. Such patients usually recover spontaneously or under simple surgical management supplemented, when indicated, by incision and drainage. The addition of chemotherapy may have considerable bearing on the invasiveness of the infective agent and on the rate of healing or on the necessity for surgical interference, but this becomes a difficult matter to evaluate in any single case or in any group of cases. Moreover, some evidence of invasiveness as indicated by lymphangitis, or bacteremia, or both, while generally indicative of severe infection, is frequently followed by rapid defervescence in the absence of chemotherapy and under only simple and conservative management. This occurs frequently enough to warrant caution in ascribing beneficial effects to antibiotic treatment in any given case merely because the antibiotic was used.

In severe cases with bacteremia, particularly those with deep-seated infections, whether they be in the lung, in bone, in body cavities, or in deep tissues, the evaluation of therapy is even more difficult. In these cases it is well recognized, as both a medical and surgical principle, that treatment with a specific antibacterial agent, in order to be effective, must be early, intensive, and prolonged. This is particularly true where areas of suppuration have already developed and have localized, in situations where they are not readily accessible to evacuation, or where the condition of the patient does not warrant immediate exploration. Under such conditions, particularly where the causative organisms are not available for study, treatment must be started empirically. By the time the organisms become available, there may be a change for the better or worse in the condition of the patient.

It takes a certain amount of experience, courage, and tenacity to persist in the treatment regimen originally outlined when, for example, the results of a culture from the blood or drainage site or from a site other than that of the principle lesion yields an organism that, by the tests used, must be interpreted as insensitive to the antibiotics that are being given at the time, even if the condition of the patient indicates a favorable response to treatment. It is well known, moreover, that even in infections where organisms are sensitive and where, either because other antibiotics are not available at the time or because the organism is found to be highly susceptible, treatment is continued, severe symptoms and even the bacteremia may persist for several days, and

clinical improvement may nevertheless follow, although it may be very slow. Eventually, recovery may be complete without change in therapy. If the organisms are insensitive to the agents being used it is difficult for most physicians to resist changing the therapeutic regimen, even in spite of improvement on the part of the patient that suggests that a therapeutic effect is being achieved. This is all the more true if there is no clinical improvement, if bacteremia persists, or if the lesion appears to be progressing. Nevertheless, in many instances where the change is not made, either because no other available agent appears to be effective *in vitro* against the organism that was tested or for other reasons, the patients may go on to uneventful recovery.

Many case reports could be cited from the clinical material just reviewed that would illustrate these and other problems arising in the course of evaluating chemotherapeutic agents in staphylococcal infections. This, however, would not be fruitful in relation to the over-all purpose of this monograph, nor would it be particularly rewarding to cite, in detail, reports from the literature, of which there are, of course, many. On the whole, the great majority of such reports have dealt with individual cases or with small groups of cases from which generalizations may be difficult. Another large number of reports has dealt with special groups of cases such as pleural and pulmonary infections, osteomyelitis, or endocarditis, and among these are very few recent reports in which adequate advantage has been taken of all of the currently available agents used under proper bacteriologic control. Finally, there is a sizable number of papers intended particularly to bring out the effects of individual antibiotics, particularly new ones that are being investigated.

Perhaps 1 important point looms up from these reports that may be worth mentioning, even though it may not be entirely proper to do so here, since this paper was, perhaps, expected to dwell primarily on the efficacy of other and newer agents. This point is so striking, however, although now so frequently neglected when dealing with staphylococcal infection, that it should be mentioned. It is the high degree of efficacy of penicillin when properly used with respect to dosage regimen, duration of therapy, and in the proper types of cases, particularly those with submerged infections due to initially sensitive strains. This was brought out recently by Fisher, Wagner, and Ross[2] in their report on staphylococcal endocarditis from Johns Hopkins Hospital, Baltimore, Md. It is also noteworthy in the many reports of cases of acute primary hematogenous osteomyelitis.

The effectiveness of penicillin in early cases of primary hematogenous osteomyelitis cannot be overemphasized, and it is noteworthy that, in the great majority of patients now admitted with this type of lesion, the organisms are still susceptible to penicillin and yield to treatment without surgery. It appears important to avoid surgery unless abscess formation with pressure has already taken place, and even then to invoke a minimum of surgical intervention, limited to simple drainage, every effort being made to avoid contamination from without. The greatest danger is from introducing new and resistant strains from the contaminated environment.

Resistance to antibiotics. The development of resistance in originally sensitive strains in deep lesions, as brought out in other papers and discussions in

this monograph, is probably not an important problem, provided that the lesion does not have access to contamination with new and resistant organisms. When a systemic infection has originated from a surface lesion that is still accessible to such contamination, the problem of antibiotic resistance of the new strains may assume major importance.

The problem of antibiotic-resistant staphylococci has been touched upon only casually. A review of the literature on this subject that was presented in considerable detail elsewhere[3] has yielded only hints here and there as to possible methods for the control of the spread of such strains. Unfortunately, although a number of methods have yielded some measure of temporary success, there is as yet no real evidence that any of these measures has brought a lasting solution to this problem. No clear solution has been advanced to the problem of chemotherapeutic management of serious infections caused by such antibiotic-resistant strains, except by careful individualization with intensive bacteriologic control.

Because prolonged and intensive therapy is required to eliminate staphylococci from established lesions and, either because staphylococci are genetically more vulnerable than many other species to the development of increased resistance or because resistant variants are already available in greater numbers than among many other species of pyogenic organisms, the problem of emergence of resistant strains of staphylococci during antibiotic therapy may assume considerable importance. In instances of infections that are not accessible to contamination by new strains from without and where the infection may be presumed to be due to a single strain, this problem can probably be coped with adequately by the use of combinations of antibiotics in much the same manner as in the treatment of tuberculosis. Observations *in vitro*[3] have indicated that combinations of antibiotics, each of which is initially active, may delay the emergence of resistance and keep it at a lower level when it occurs. While this may be expected in clinical therapy, confirmation in large numbers of patients is needed, and optimum dosage regimens for such combinations need to be determined.

Relation to surgery. Finally, it is worth noting that staphylococcal infections, which historically have fallen within the province of the surgeons, like many other types of infections formerly within their domain, have suffered to some extent by the dichotomy of the types of therapy available for the management of such cases. This has resulted, on the one hand, in a failure on the part of many surgeons to recognize the full potentialities of conservative, nonsurgical, treatment which the rational use of antibacterial agents permits and, on the other hand, in either the persistence of the medical man in continuing treatment in the face of obvious failure or, on the other extreme, in his willingness to give up too soon when faced with pressure from impetuous surgical colleagues. It is this state of affairs that still permits many otological surgeons to perform large numbers of mastoidectomies, and thoracic or general surgeons to deprive many patients of part of one or more of their ribs, while others among their colleagues, with at least equal skill but with more patience and perseverance, rarely find it necessary to perform such operations except under unusual, specific, and well-defined circumstances.

In the case of staphylococcal infections this is perhaps most evident with respect to the management of cases of acute hematogenous osteomyelitis in which failure to recognize the normal evolution of the process under properly administered chemotherapy has resulted in operative interference under conditions in which complete recovery without such intervention, and with eventual complete restoration of function and normal anatomic structure, could have been expected with continued and intelligently directed chemotherapeutic management alone. Surgical interference under such circumstances has resulted, in many instances, in secondary invasion with resistant organisms that have altered the prognosis with respect to early or complete recovery and have necessitated the use of many other agents that would otherwise not have been required.

Fortunately, there has emerged in the antimicrobial era a group of surgeons who have become intimately concerned with evaluation of the potentialities of such agents in limiting the requirements for surgery or, more properly stated, in delineating the scope of surgical and conservative antimicrobial therapy in the best interest of the patient. In certain staphylococcal infections, as already intimated, there are recognized indications for well-timed surgical intervention that may spell the difference between success and failure of effective chemotherapeutic agents. In others of these infections such indications could be worked out by careful observations in large numbers of patients with properly controlled studies.

Adrenocortical hormones. Finally, some mention may be made of the use of adrenocortical steroids in staphylococcal infections. Many cases have now been encountered in which disseminated and rapidly fatal staphylococcal infections have occurred in patients while they were under treatment with cortisone for such serious skin diseases as exfoliative dermatitis and pemphigus, and sometimes for less serious illness in which the indications are far from clear. In some of the patients, however, fulminating staphylococcal infections have followed the use of cortisone in the treatment of shock from various causes or in serious infections other than staphylococcal where the indication was doubtful. The effect of cortisone on the "toxemia" of severe staphylococcal infection cannot be evaluated from the data available thus far. The findings in some of the fatal cases in which it was used suggest that it may have contributed to an aggravation of the infection. Without more detailed study, however, this is as difficult to assess as are the alleged benefits ascribed by others to its use in cases that progressed favorably.

Comment

The observations presented here were made as part of a series of studies designed to determine the real magnitude and clinical significance of the staphylococcal infections that are being encountered. It is hoped, of course, that these, together with the many other studies currently in progress in many clinics and laboratories will in turn permit a more rational approach to the problems of management and control of such infections. Obviously the whole problem is a dynamic one, but it is difficult, from available data, to determine to what extent staphylococcal infections have been altered by the availability

and widespread use of increasing numbers of highly active antimicrobial agents. Comparisons of the data presented here with those from other sources are not justified, and may be misleading unless very similar types of material are considered.

In addition to more detailed studies on several aspects of the material presented, it is planned to obtain similar data from this hospital from previous years in order to get a better view of the over-all picture as it has developed. Only when such data have become available and have been properly analyzed will it be possible properly to assess the material presented here and to determine whether staphylococcal infections have actually increased in incidence, severity, and resistance to available methods of treatment, including antimicrobial therapy. Unfortunately, the large amount of valuable literature dealing with the antibiotic resistance of staphylococci that has been accumulated in recent years, although it has shed much light on the epidemiology of staphylococcal infections, has not brought adequate answers to these equally important questions.

Summary

Data have been presented on the kinds of staphylococcal infections currently being encountered at the Boston City Hospital. Some of the problems involved in the evaluation of antimicrobial therapy in such infections have been discussed.

Acknowledgment

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STUDIES ON STAPHYLOCOCCI FROM HOSPITAL PATIENTS: II. EFFECT OF ANTIMICROBIAL THERAPY AND HOSPITALIZATION ON CARRIER RATES*

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We have reported that the staphylococcal flora of a hospital patient population was characterized by a high predominance of strains that were lysed by Group III staphylococcal bacteriophages and were resistant to several antimicrobial drugs.[1] This study revealed that new patients did not carry significant numbers of staphylococci of these characteristics, but that the staphylococci did appear in cultures taken during hospitalization, at rates that were highest among patients who were receiving tetracyclines, lower among patients receiving penicillin, and least when no treatment was given. In the demonstration of this remarkable qualitative alteration in staphylococcal flora, sufficient data were not obtained to learn whether there were associated quantitative changes in the staphylococcal carrier state.

For this reason a further investigation was made that was designed to verify the existence of the phenomena already described and to provide data sufficient to evaluate the carrier state in the presence of these changes. The results of this latter investigation confirmed the findings of the earlier report, namely, that there existed a reservoir of phage Group III multiple drug-resistant staphylococci in hospital patients and that organisms with these characteristics appeared in cultures from new patients under conditions previously described. In addition, cultures were taken frequently enough to permit an estimate of the possible effect of antimicrobial therapy and hospitalization on carrier rates.

In the present report consideration will be given to the questions relating to carrier rates and, in a subsequent communication,[2] the observations confirmatory of the previous studies of antimicrobial susceptibility and phage grouping will be reviewed.

Materials and Methods

Source of Patient Subjects

The patients on a 30-bed medical ward of the United States Veterans Administration Hospital, Nashville, Tenn., were selected for study. Nose and throat cultures for staphylococci were made daily, Monday through Friday, except holidays, each week, from January 24 to June 17, 1955. An attempt was made to obtain a culture from every patient each day. This effort was about 90 per cent effective. In the course of the study 227 different cases were cultured that, except for 5 readmissions, represented different patients, all but 1 of which were adult males. Many of the patients were not acutely ill, and chronic cardiac disease, often with decompensation, chronic pulmonary disease,

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peptic ulcer, neoplasia, and cirrhosis were the most frequent diagnoses. Clinical information and a record of doses of antimicrobial drugs were collected each day by a member of the investigating team on all patients from whom cultures were taken. Patients who received antimicrobial treatment were usually ill with some type of pulmonary infection.

Bacteriological Procedures

Collection of specimens. Cultures were made with sterile, cotton-tipped swabs, moistened with sterile broth just before use. The same swab was inserted about 1 inch into each nostril for nose cultures, and a separate swab was streaked over the lateral and posterior pharyngeal walls for throat cultures. After use, the swabs were inserted into tubes containing a small volume of sterile broth and were promptly returned to the laboratory. Each nose and throat swab was streaked over the surface of one half a culture plate, except for a short period during the study when a loopful of broth from the carrying tube was used as inoculum. After overnight incubation, colonies microscopically and macroscopically resembling staphylococci were tested for fermentation of mannitol, hemolysis of sheep's blood, and coagulase production. Pigmentation was recorded from the original culture plate. The coagulase test was performed by the method of Chapman *et al.*,[3] using a loopful of surface growth in one-half ml. of blood-bank plasma. Tests were incubated at 37° C. in a water bath and read at intervals for 3 hours. Tests with negative readings were again examined after overnight incubation.

As a policy of the study only 1 staphylococcal colony from each culture was preserved, with preference being given to coagulase-producing strains. Very frequently 2 or more colonies were subcultured, however, in an effort to detect coagulase-positive staphylococci.

Primary isolation was carried out on 0.02 per cent sodium azide blood agar* in the period January 24 to March 1, 1955, except for a few days when phenylethyl-alcohol medium was used. After March 1 the concentration of sodium azide was reduced to 0.01 per cent and this was used until April. At this point the basal peptone medium,† without inhibitors but with 5 per cent human blood, was used until May 25, when 0.02 per cent sodium azide medium was again employed.

The effectiveness of sodium azide blood agar and the basal peptone blood agar in the isolation of coagulase-positive staphylococci was compared by inoculating each of a large number of nose and throat swabs obtained in the study on one half a plate of each medium. Later, a similar study was performed comparing sodium azide blood agar with Chapman-Stone medium,‡ using swabs obtained during a different period of the study. The results of these 2 comparisons may be seen in TABLES 1 and 2.

Cultures on azide medium and peptone medium (TABLE 1) yielded 7.6 and 10.1 per cent, respectively, containing coagulase-positive staphylococci. Only 5.4

* Sodium azide (0.02 per cent) agar medium obtained from the Difco Laboratories, Inc., Detroit, Mich. Five per cent citrated human blood was added before use.

† Peptone medium—trypticase soy broth, Baltimore Biological Laboratories, Baltimore, Md., or tryptic soy broth, Difco). The medium contains an enzymatic digest of casein and soya meal. Five per cent blood was added.

‡ Chapman-Stone medium for isolation of staphylococci. This medium contains 8.5 per cent sodium chloride. Obtained from the Difco Laboratories, Inc., Detroit, Mich.

TABLE 1

COMPARISON OF AZIDE BLOOD AGAR WITH PEPTONE BLOOD AGAR IN THE PRIMARY ISOLATION OF COAGULASE-POSITIVE STAPHYLOCOCCI

Number of cultures	Azide blood agar *positive* / Peptone blood agar *positive*		Azide blood agar *positive* / Peptone blood agar *negative*		Azide blood agar *negative* / Peptone blood agar *positive*		Total azide blood agar *positive*		Total peptone blood agar *positive*	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
277	15	5.4	6	2.2	13	4.7	21	7.6	28	10.1

per cent of the swabs, however, gave positive cultures on both the media, with 2.2 and 4.7 per cent appearing on azide blood agar or peptone blood agar only. The total yield of different positive cultures from both media was accordingly 12.3 per cent. A similar result was observed in the comparison with Chapman-Stone medium (TABLE 2). The total yield of different positive cultures on both media was 16.4 per cent. These comparisons suggest that the differences in the effectiveness of these media in the isolation of coagulase-positive staphylococci are not large and that the use of 2 kinds of media, or 2 independent uses of the same medium, might increase the yield of staphylococci by 25 to 50 per cent. Comparisons further suggest that the true detectable yields by these methods are no more than double the rates found by a single culture.

Cultural and biochemical reactions of the staphylococci isolated in the study are as follows:

Coagulase-positive staphylococci. Among 649 strains studied, 85.2 per cent (553 strains) were hemolytic, 95.8 per cent (622) fermented mannitol, 74.3 per cent (482) were gold or orange colored, 18.6 per cent (121) were cream colored, and only 7.1 per cent (46) were nonpigmented (white).

Coagulase-negative staphylococci. Among 2210 strains studied, 75.5 per cent (1669 strains) were hemolytic (any degree of observable hemolysis was con-

TABLE 2

COMPARISON OF AZIDE BLOOD AGAR WITH CHAPMAN-STONE MEDIUM IN THE PRIMARY ISOLATION OF COAGULASE-POSITIVE STAPHYLOCOCCI

Number of cultures	Azide blood agar *positive* / Chapman-Stone agar *positive*		Azide blood agar *positive* / Chapman-Stone agar *negative*		Azide blood agar *negative* / Chapman-Stone agar *positive*		Total azide blood agar *positive*		Total Chapman-Stone agar *positive*	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
391	22	5.6	23	5.9	19	4.9	45	11.5	41	10.5

sidered as a positive test), 10.9 per cent (243) fermented mannitol, 61.0 per cent (1370) were nonpigmented, 24.6 per cent (544) were cream colored, and 13.3 per cent (296) were gold to orange colored.

Results

The carrier rates of coagulase-positive and coagulase-negative staphylococci in the nose and throat and the proportion of patients receiving antimicrobial drugs during the 5½ months of the study are described in FIGURE 1, and in TABLES 3, 4, and 5. Coagulase-positive staphylococci were found most frequently in nose cultures in percentages by weeks, ranging from 8 to 36 with a mean of 19.2. A total of 2439 cultures was taken during the whole study, the numbers each week ranging from 86 to 135. Approximately the same number of throat cultures was taken, but the weekly percentage rates for coagulase-positive staphylococci were lower, ranging from 1.9 to 14.8 per cent, with a mean of 7.5 per cent.

Coagulase-negative staphylococci were much more frequent, especially in nose cultures, where an average of 73.3 per cent positive cultures was observed. This figure was lower than the number that actually occurred since, in many cultures containing coagulase-positive staphylococci, coagulase-negative strains were also isolated, although they were not preserved for the study. Coagulase-negative organisms were isolated less frequently in throat cultures, with a mean rate of 17.8 per cent observed. Because of the high frequency of occurrence of coagulase-negative staphylococci in the nose and the alteration in the true

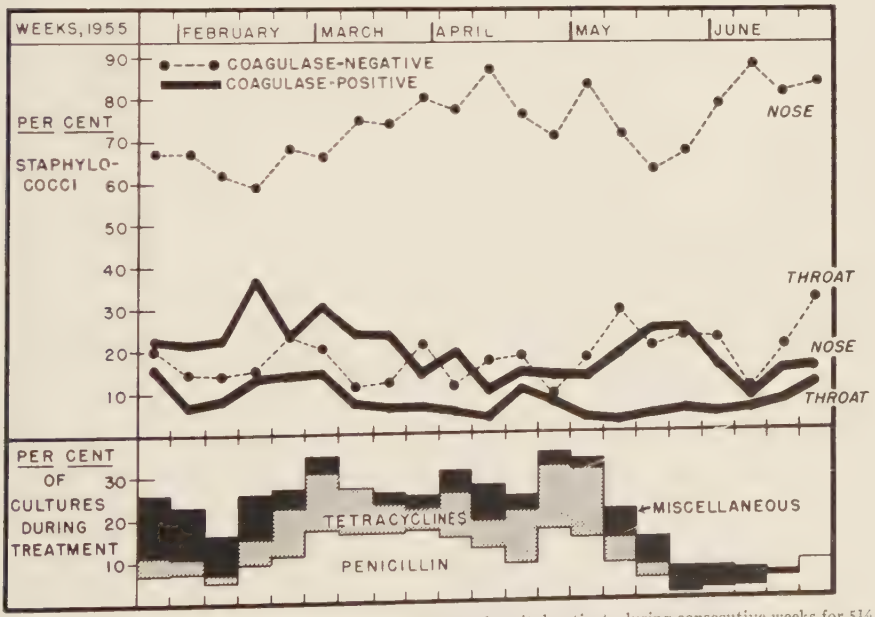

FIGURE 1. Survey of staphylococcal carrier state among hospital patients during consecutive weeks for 5½ months. Kind and amount of antimicrobial therapy indicated at bottom of figure

TABLE 3

CARRIER RATES OF STAPHYLOCOCCI IN THE NOSES OF HOSPITALIZED PATIENTS

Week (1955)	Total number of cultures	Coagulase-positive		Coagulase-negative	
		Number	Per cent	Number	Per cent
Jan. 24	135	30	22.2	90	66.7
Jan. 31	130	27	20.8	87	66.9
Feb. 7	111	24	21.6	69	62.2
Feb. 14	115	41	35.7	68	59.1
Feb. 21	96	23	24.0	65	67.7
Feb. 28	125	37	29.6	84	67.2
Mar. 7	114	27	23.7	85	74.6
Mar. 14	127	29	22.8	94	74.0
Mar. 21	127	18	14.2	101	79.5
Mar. 28	128	24	18.8	98	76.6
Apr. 4	122	12	9.8	106	86.9
Apr. 11	130	18	13.8	99	76.2
Apr. 18	114	15	13.2	81	71.1
Apr. 25	124	16	12.9	103	83.1
May 2	106	19	17.9	75	70.8
May 9	123	30	24.4	78	63.4
May 16	120	29	24.2	80	66.7
May 23	94	14	14.9	73	77.7
May 30	86	7	8.1	76	88.4
June 6	108	15	13.9	88	81.5
June 13	104	15	14.4	87	83.7
Total	2439	470	19.2	1787	73.3

TABLE 4

CARRIER RATES OF STAPHYLOCOCCI IN THE THROATS OF HOSPITALIZED PATIENTS

Week (1955)	Total number of cultures	Coagulase-positive		Coagulase-negative	
		Number	Per cent	Number	Per cent
Jan. 24	128	19	14.8	26	20.3
Jan. 31	129	8	6.2	18	14.0
Feb. 7	111	9	8.1	15	13.5
Feb. 14	116	15	12.9	17	14.7
Feb. 21	88	12	13.6	20	22.7
Feb. 28	125	18	14.4	25	20.0
Mar. 7	110	8	7.3	12	10.9
Mar. 14	127	8	6.3	15	11.8
Mar. 21	126	8	6.3	27	21.4
Mar. 28	126	6	4.8	14	11.1
Apr. 4	120	4	3.3	21	17.5
Apr. 11	129	13	10.1	23	17.8
Apr. 18	113	8	7.1	10	8.8
Apr. 25	120	4	3.3	20	16.7
May 2	103	2	1.9	30	29.1
May 9	121	5	4.1	24	19.8
May 16	118	6	5.1	27	22.9
May 23	92	4	4.3	20	21.7
May 30	85	4	4.7	9	10.6
June 6	105	7	6.7	21	20.0
June 13	101	11	10.9	31	30.7
Total	2393	179	7.5	425	17.8

TABLE 5

PER CENT OF NASAL CULTURES FOR STAPHYLOCOCCI TAKEN DURING PENICILLIN (ALONE OR WITH STREPTOMYCIN), TETRACYCLINE, OR OTHER COMBINATIONS OF ANTIBIOTIC TREATMENT

Week 1955	Total number of cultures	Cultures during penicillin treatment		Cultures during tetracycline treatment		Cultures during other treatment	
		Number	Per cent	Number	Per cent	Number	Per cent
Jan. 24	135	10	7.4	6	4.4	19	14.1
Jan. 31	130	10	7.7	5	3.8	15	11.5
Feb. 7	111	5	4.5	2	1.8	11	9.9
Feb. 14	115	10	8.7	7	6.1	13	11.3
Feb. 21	96	10	10.4	11	11.5	5	5.2
Feb. 28	125	21	16.8	17	13.6	5	4.0
Mar. 7	114	18	15.8	13	11.4	0	0.0
Mar. 14	127	20	15.7	9	7.1	4	3.1
Mar. 21	127	21	16.5	7	5.5	4	3.1
Mar. 28	128	19	14.8	14	10.9	7	5.5
Apr. 4	122	15	12.3	9	7.4	10	8.2
Apr. 11	130	11	8.5	17	13.1	4	3.1
Apr. 18	114	19	16.7	18	15.8	3	2.6
Apr. 25	124	18	14.5	21	16.9	3	2.4
May 2	106	9	8.5	6	5.7	8	7.5
May 9	123	6	4.9	3	2.4	9	7.3
May 16	120	1	0.8	0	0.0	8	6.7
May 23	94	2	2.1	0	0.0	5	5.3
May 30	86	2	2.3	0	0.0	4	4.7
June 6	108	5	4.6	0	0.0	1	0.9
June 13	104	9	8.7	0	0.0	0	0.0
Total	2439	241	9.9	165	6.8	138	5.7

rates resulting from discarding those cultures found in the presence of coagulase-positive strains, a full analysis of these data was not made.

Nose cultures taken from patients on days when they were receiving antimicrobial treatment were enumerated, and they are shown at the lower portion of FIGURE 1 and TABLE 5 as the per cent of all cultures taken during each week. This provides an index of the use of antimicrobial therapy in the study. Penicillin was the most frequently used antimicrobial drug, and it was given most intensively during March, April, and the first half of May, when approximately 15 per cent of cultures each week were obtained from patients treated with this agent. Some of the patients receiving penicillin also received streptomycin, which is not otherwise indicated. Tetracyclines were given to a lesser extent, most frequently, however, during the period of heavy use of penicillin. Miscellaneous regimens that included sulfonamides and combinations of several currently available antimicrobial drugs were given to a small percentage of patients. There does not appear to be any gross evidence of correlation of carrier rates with the intensity of use of antimicrobial drugs.

It was observed that patients with high nasal carrier rates tended to have high throat carrier rates. This relationship is shown graphically in FIGURE 2, based on data from the 83 patients in the study from whom cultures were taken on 10 or more days. The percentage of positive nose cultures appears on the abscissa, and the percentage of positive throat cultures is shown on the ordi-

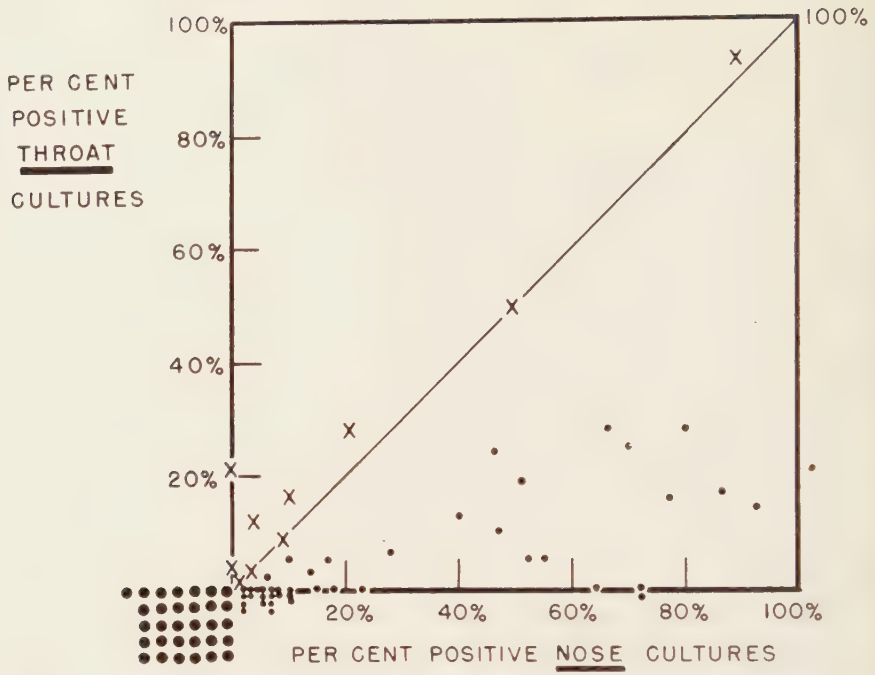

FIGURE 2. Correlation of nose and throat carrier rates in the same 83 patients. Nose cultures were more frequently positive, and throat carrier rates tended to increase in proportion to nose carrier rates.

nate. Each plotted point represents the mean nose and throat carrier rate for coagulase-positive staphylococci of 1 patient. From this figure it is apparent that a moderate positive association exists between the nose and throat finding rates. Finding rates in the throat that are higher than in the nose are exceptional. Ten of the 52 patients who had some positive cultures had percentages of positive throat cultures as high or higher than those from the nose. These are marked by X's in the chart. It is suspected that these patients were exceptional because of the presence of some lesion, infectious or otherwise, that encouraged the growth of staphylococci in the throat. Examination of their clinical records, however, failed to show the presence of lesions to which this effect could readily be assigned.

The group of 83 patients referred to in FIGURE 2 consisted of 17 patients who were already present in the hospital when the study was begun and 66 who were admitted during the course of the study. In TABLE 6 the carrier rates of the 66 patients are presented according to the percentage of nasal cultures containing coagulase-positive staphylococci. The purpose of this analysis is to show the frequency of occurrence of rates of various degrees of positiveness, and to correlate them with the frequency of use of antimicrobial drugs. It will be seen that 28 (42.4 per cent) were consistently negative. Fifteen (22.7 per cent) had rates in the range of 2 to 9 per cent, 8 (12.1 per cent) in the range of 10 to 19 per cent, while the remaining 15 (22.7 per cent) showed an increasingly frequent occurrence up to a high value of 89 per cent. The number of cultures

TABLE 6

CARRIER RATES OF COAGULASE-POSITIVE STAPHYLOCOCCI IN THE NOSES OF HOSPITAL PATIENTS CULTURED 10 OR MORE DAYS FROM ADMISSION

Percentage of cultures positive	Patients cultured		Mean number of cultures per patient	Patients receiving antimicrobial treatment	
	Number	Per cent		Number	Per cent
All negative	28	42.4	18	7	25.0
2–9	15	22.7	34	7	46.6
10–19	8	12.1	19	3	37.5
20–29	2	22.7	24	6	40.0
30–49	3				
50–69	4				
70–89	6				
Total	66	100.0	22.9	23	34.8

that were the basis of these determinations was reasonably uniform from group to group, with a mean value of 22.9 per cent.

The percentage of patients in the groups that received antimicrobial therapy ranged from 25.0 to 46.6 per cent, with a mean percentage of treated patients for the group of 34.8. The proportion of patients with high carrier rates who received antimicrobial therapy was not significantly different from the percentage of treated patients in the other groups.

The sequence of positive and negative nose cultures for coagulase-positive staphylococci among the 66 patients was examined in an effort to recognize any characteristic alterations occurring in the course of hospitalization.

In addition to the 28 patients with all cultures negative, the 23 patients with carrier rates ranging from 2 to 19 per cent were so infrequently positive that no pattern could be distinguished. For example, 1 patient from whom cultures were obtained on 17 days had positive nasal cultures only at 9, 16, and 17 days.

Among the 15 high-rate carriers (20 to 89 per cent) it appeared that, once established, the carrier state continued to be characterized by frequent positive cultures (usually positive at least every other day) throughout the remaining period of observation. Thus 9 of these carriers had their first positive culture on their first- or second-hospital day while 1 each were first positive on days 3, 4, 4, 8, 12, and 32. In view of the relative inefficiency of the sampling procedure, it can be stated with confidence only that the patients with the first positive cultures on the eighth day or thereafter represented conversion to the carrier state during hospitalization. Thus 3 of 66 patients (4.5 per cent) appeared to become carriers after hospital admission. It should be emphasized that it is possible that among patients with low carrier rates conversion to a carrier state may have occurred, but definite evidence of this was not obtained by the criteria used in this study.

Finally, an estimate was made of the net quantitative effect of the various factors related to hospitalization, including conversion to the carrier state, on the mean carrier rate of the 66 patients. The percentages of positive nose and throat cultures obtained during the first 5 days and last 5 days of culturing were

TABLE 7

PER CENT OF PATIENTS WHO WERE CARRIERS AND PER CENT OF NOSE AND THROAT CULTURES CONTAINING COAGULASE-POSITIVE STAPHYLOCOCCI DURING FIRST 5 AND LAST 5 DAYS OF CULTURES

	First 5 days*				Last 5 days*			
	Patients with 1 or more pos. cult.		Cultures positive		Patients with 1 or more pos. cult.		Cultures positive	
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent
Nose	21	31.8	48	14.5	23	34.8	55	16.6
Throat	7	10.6	8	2.4	12	18.1	16	4.8

* First 5 or last 5 days cultured; 330 nose and throat cultures each period from a total of 66 patients.

determined for each patient. These periods represent almost invariably the first 5 to 7 or last 5 to 7 days of the patients' stay on the ward. The data are shown in TABLE 7 and reveal that an increase occurred in the per cent of positive nose and throat cultures, and the percentage of patients who were carriers in the last 5 days over the first 5 days.

The 66 patients whose cultures were the basis for the preceding analyses were selected because they had been cultured on 10 or more days during a period of at least 2 weeks of hospitalization. Because of the possibility that these 66 patients might react differently from patients with short term illnesses, the per cent of positive nose cultures was determined for all patients in the study by consecutive 10-day periods of hospitalization. In addition, the per cent of positive cultures in untreated patients and in those during and after antimicrobial therapy was separately determined for 2 consecutive 30-day periods. These data appear in TABLE 8 and reveal, for a period covering 6 consecutive 10-day periods, that there is no appreciable increase in the percentage of posi

TABLE 8

PER CENT INCIDENCE OF COAGULASE-POSITIVE STAPHYLOCOCCI IN NOSE CULTURES BY DAY IN HOSPITAL

Day in hospital	All cases			No treatment			During or after antimicrobial treatment			No. cases cultured
	Total cultures	No. positive	Per cent	Total cultures	No. positive	Per cent	Total cultures	No. positive	Per cent	
1–10	810	127	16							
11–20	575	77	13	1272	198	15.5	477	71	14.8	227
21–30	364	65	18							
	1749	269	15.4							
31–40	182	37	20							
41–50	154	26	17	256	52	20.3	178	28	15.7	39
51–60	98	17	17							
	434	80	18.4							

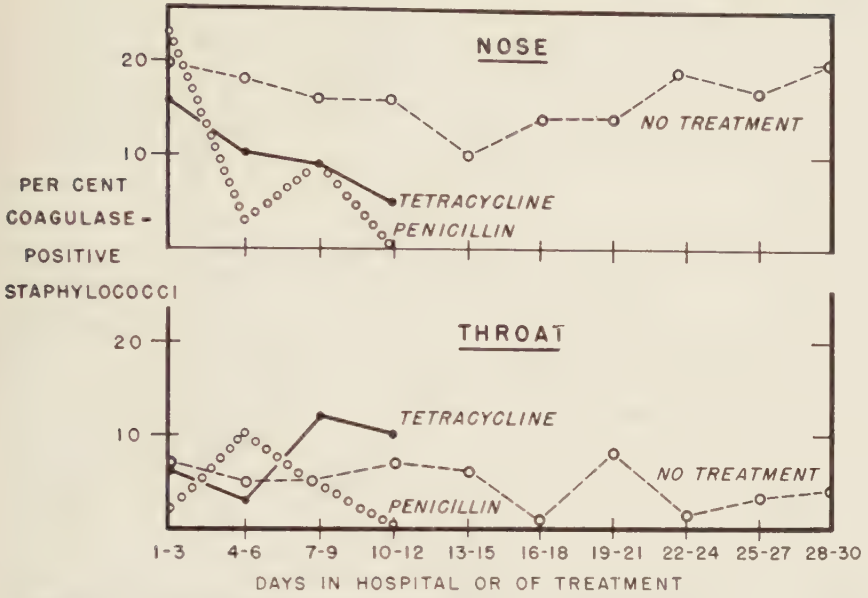

FIGURE 3. The percent of coagulase-positive cultures of the nose diminished under penicillin and tetracycline treatment. Among untreated patients nasal cultures remained at a relatively uniform rate (12 to 20 per cent) and among throat cultures there was little change during hospitalization, with or without treatment.

tive nose cultures with values ranging from 13 to 20 during the six 10-day periods. The percentage of positive nose cultures in untreated patients increased from 15.5 to 20.3 per cent in consecutive 30-day periods and increased from 14.8 to 15.7 per cent among treated patients.

The effect of antimicrobial therapy and hospitalization on the carrier state was finally evaluated by determining the per cent of nose and throat cultures with coagulase-positive staphylococci in the course of therapy with the tetracyclines, penicillin (some patients also received streptomycin), or no treatment. Only cultures taken on days when a drug was administered to the treated group were used in the determinations. These data appear in FIGURE 3 and in TABLES 9 and 10.

The data are grouped in consecutive 3-day intervals. During the first 3 days, treated and untreated patients showed nasal carrier rates that ranged around 20 per cent. In the course of 12 days of treatment there was a gradual decline in the percentage of positive cultures in the treated groups, in contrast to the untreated group. The mean per cent of positive cultures for the 30-day period of observation was 16.3 per cent among untreated patients, while the mean per cent of positive throat cultures in the treated and untreated groups fluctuated around 5 per cent.

DISCUSSION

In a 5½-month study, observations were made on the effect of hospitalization and antimicrobial therapy on the staphylococcal carrier rates of 222 hos-

TABLE 9

CARRIER RATE OF STAPHYLOCOCCI IN THE NOSES OF HOSPITALIZED PATIENTS DURING ANTIMICROBIAL THERAPY OR NO THERAPY

Treatment day	Total cultures	Coagulase-positive		Coagulase-negative	
		Number	Per cent	Number	Per cent
Tetracycline					
1–3	32	5	15.6	26	81.2
4–6	30	3	10.0	25	83.3
7–9	34	3	8.8	29	85.3
10–12	22	1	4.5	18	81.8
1–12	118	12	10.2	98	83.1
Penicillin					
1–3	26	6	23.1	16	61.5
4–6	29	1	3.4	28	96.6
7–9	21	2	9.5	16	76.2
10–12	14	0	0.0	14	100.0
1–12	90	9	10.0	74	82.2
No treatment					
1–3	200	40	20.0	142	71.0
4–6	207	37	17.9	156	75.4
7–9	203	23	11.3	168	82.8
10–12	145	23	15.9	110	75.9
13–15	141	14	9.9	113	80.1
16–18	95	13	13.7	74	77.9
19–21	90	13	14.4	65	72.2
22–24	74	14	18.9	54	73.0
25–27	67	11	16.4	51	76.1
28–30	50	10	20.0	38	76.0
1–30	1272	198	16.3	971	76.3

pital patients. The results reveal that carrier rates of coagulase-positive staphylococci in nose cultures showed a slight increase during hospitalization if observations were made over a 60-day period. This increase occurred in patients given antimicrobial drugs and in those who were untreated. A similar slight increase was observed in the proportion of positive nose and throat cultures and in the proportion of patients who were carriers if a series of cultures obtained near the time of discharge from the ward was compared to admission cultures. In shorter periods of observation (30 days) no such increase was observed among cultures from untreated patients, nor in treated patients observed for 12 days. In the latter group there was a slight decrease in nasal carrier rates.

The characteristics of the carrier state were examined and it was found that a small percentage of the patients constituted a high carrier-rate group that provided most of the positive cultures. These patients were usually car-

TABLE 10

CARRIER RATE OF STAPHYLOCOCCI IN THE THROATS OF HOSPITALIZED PATIENTS DURING ANTIMICROBIAL THERAPY OR NO THERAPY

Treatment day	Total cultures	Coagulase-positive		Coagulase-negative	
		Number	Per cent	Number	Per cent
Tetracycline					
1–3	31	2	6.5	4	12.9
4–6	30	1	3.3	7	23.3
7–9	34	4	11.8	4	11.8
10–12	20	2	10.0	8	40.0
1–12	115	9	7.8	23	20.0
Penicillin					
1–3	26	1	3.8	10	38.5
4–6	29	3	10.3	3	10.3
7–9	21	1	4.8	11	52.4
10–12	14	0	0.0	3	21.4
1–12	90	5	5.6	27	30.0
No treatment					
1–3	197	15	7.6	29	14.7
4–6	202	11	5.4	28	13.9
7–9	197	10	5.1	32	16.2
10–12	144	7	4.9	27	18.8
13–15	135	8	5.9	18	13.3
16–18	94	1	1.1	18	19.1
19–21	85	7	8.2	8	9.4
22–24	73	1	1.4	13	17.8
25–27	62	2	3.2	12	19.4
28–30	50	2	4.0	4	8.0
1–30	1239	64	5.2	189	15.2

riers on admission and remained fairly persistently positive throughout hospitalization.

If the finding of a considerable degree of stability of carrier rates for coagulase-positive staphylococci in hospitalized persons, with or without antimicrobial therapy, occurred generally, it would suggest that the factors that lead to the establishment and perpetuation of the carrier state are different from those that cause the concurrent pronounced changes in drug-susceptibility patterns and phage groupings referred to earlier. Evidence from many sources suggests that drug resistance and its attendant phenomena are a consequence of antimicrobial treatment. Since changes in drug resistance and phage grouping appear to be dissociated from alterations in the carrier rates, it seems possible that the antimicrobial regimens studied have little influence on carrier rates of coagulase-positive staphylococci in the nose and throat. It is suggested that the essential control of the carrier state resides in factors present in the animal

host that, for short periods at least, are relatively independent of environmental influences. This concept is not intended to apply to all antimicrobial drugs, since agents highly and uniformly active against staphylococci, such as erythromycin, have greatly reduced the frequency of positive nose and throat cultures in small studies made by us. At present, however, this point seems less important than the fact that carrier rates for staphylococci did not increase among patients treated with the 2 antimicrobial regimens, for it would be in the latter situation that an implication of increased virulence and threat of infection would reside.

It has been our concept that the shifts from 1 kind of *Staphylococcus* to another among carriers actually represented "replacement" of staphylococci residing on mucous membranes with others from the environment. This concept appears to be based on sound epidemiologic grounds in that the change in characteristics occurs too rapidly for selection of resistant mutants *in vivo*, and the environment is filled with staphylococci with appropriate characteristics ready for acquisition. An alternative explanation is available, however, to explain the apparent replacement, based on the work of Gould.[4] This investigator has reported that, in the production of penicillin-resistant staphylococci by serial passages in increasing concentrations of penicillin *in vitro*, colonies of penicillinase-producing, penicillin-resistant, phage-Group III staphylococci appeared on plates originally inoculated with phage-Group I and II cultures that were susceptible to penicillin. Should this phenomenon occur *in vivo* it would satisfy many of the known facts concerning changes in the staphylococcal carrier state of hospital patients referred to previously.

Other investigators have considered the effect of hospitalization or exposure of personnel in hospital wards on staphylococcal carrier rates. Summaries of several reports are shown in TABLE 11. The reports of Rountree and Barbour,[5] Miles, Williams, and Cooper,[6] Rountree and Thomson,[7] Rountree, Freeman, and Barbour,[8] and Denton, Kalz, and Foley[9] reveal that persons who, either as employees or patients, enter into close contact with hospital patients show gradually increasing nasal carrier rates for coagulase-positive staphylococci in the course of such exposure for periods up to several weeks. The study of Miles was made in 1944, either before the availability of penicillin or while its use was very limited. His data resemble those that have been more recently reported. In 1954 Lepper and his colleagues[10] reported that patients treated with chlortetracycline showed an increase in carrier rates of staphylococci in contrast to no increase in patients treated with other drugs or who received no treatment on the same wards. Thus it would appear that the close contact inherent in hospitalization may in itself lead to from slight to moderate increases in the carrier rates for staphylococci for which due account must be taken when assessing the effect of antimicrobial therapy.

It will be apparent that although there is considerable variation in the per cent of positive cultures among the investigations described in TABLE 11, nasal carrier rates for coagulase-positive staphylococci in most of them are higher than in the present report.

The techniques of isolation and identification of staphylococci in this study have been described earlier and, compared to the methods used in the reported studies, in so far as they were described, there do not appear to be unusual dif-

TABLE 11

INCIDENCE OF COAGULASE-POSITIVE STAPHYLOCOCCI IN NOSE CULTURES

Persons cultured	Number of cultures	Cultures with coagulase-positive staphylococci		Reference
		Number	Per cent	
Student nurses entering training	127	68	53.5	Rountree and Barbour (1951)[5]
Student nurses before entering ward	116	61	52.6	
Student nurses after 5 weeks on ward	112	80	71.4	
Student nurses after 10 weeks on ward	104	71	68.3	
Outpatients	479	228	47.6	Miles, Williams, and Cooper (1944)[6]
Ward patients on admission	536	265	49.4	
Ward patients with weekly cultures	1456	794	54.5	
Nursing staff	612	392	64.1	
Blood donors	200	90	45.0	Rountree and Thomson (1949)[7]
Hospital nurses and doctors	200	105	52.5	
Blood donors	200	98	49.0	Rountree, Freeman and Barbour (1954)[8]
Patients on admission	153	52	34.0	
Patients on discharge	153	62	40.5	
Healthy school children	2762	843	30.5	Saint-Martin (1953)[11]
Second year medical students	50	10	20.0	Denton, Kalz, and Foley (1950)[9]
Science students, nurses and interns not at Maternity Center	484	139	28.7	
Fourth year medical students	50	23	46.0	
Infants, mothers, and staff at Maternity Center	375	240	64.0	
Hospitalized adults	2439	469	19.2	Present series

ferences. A possible exception is the fact that, in the present study, daily cultures were taken while, in most of the reports in TABLE 11, cultures were taken at weekly or longer intervals. It seems probable that at least a part of the differences in the yield of coagulase-positive staphylococci among the various reports, including the present one, depend upon variations in technique, frequency of sampling, or the use of different criteria in the interpretation of tests.

In contrast to the wide variation in percentage yield of coagulase-positive staphylococci from nose cultures, the yield of these organisms in throat cultures has been more uniform. Illustrative of this are data in TABLE 12 from Saint-Martin,[11] from the report of the Commission on Acute Respiratory Diseases,[12] and from the present study in which, among almost 12,000 cultures of the throat, the per cent containing coagulase-producing staphylococci varied only from 7.5 to 15 per cent.

In the isolation of staphylococci from nose and throat swabs one cannot escape the observation that large differences occur in the number of colonies of staphylococci that appear on different culture plates. In the present study heavy growth of these organisms frequently occurred from swabs from patients with high carrier rates. These observations suggest to us that the extent of

Table 12

Incidence of Coagulase-Positive Staphylococci in Throat Cultures

Persons cultured	Number of cultures	Cultures with coagulase-positive staphylococci		Reference
		Number	Per cent	
Health school children	2762	322	11.7	Saint-Martin (1953)[11]
Hospitalized young adults with acute respiratory disease	2338	349	14.9	Commission on Acute Respiratory Diseases (1947)[12]
Well army personnel	4352	406	9.3	
Hospitalized adults	2393	179	7.5	Present series

colonization of mucous membranes with staphylococci varies considerably from patient to patient. Furthermore, the differences in carrier rates that exist among the various investigations reported may reflect, to a degree, the use of procedures of different grades of sensitivity in the isolation of staphylococci. Elaborate isolation techniques might detect staphylococci even though present in small numbers, whereas simpler methods may detect staphylococci only in the more heavily positive cultures.

Quantitative differences in the positiveness of cultures have not often been considered to be of importance in epidemiologic studies on staphylococci. Wallmark[13] has reported, however, that only 61 of 182 nasal cultures containing coagulase-positive staphylococci showed heavy growth, while 44 were moderate and 77 showed sparse growth. Vogelsang,[14] in a similar study, revealed that 446 of 660 nose cultures containing coagulase-positive staphylococci showed heavy growth. It seems probable that not only are differences in the amount of growth of staphylococci in nose and throat cultures important as a cause of variation in the yield of positive cultures by different methods of procedure, but that important differences may exist in the transmissibility of staphylococci by patients, differences dependent on the extent of staphylococcal infestation in each patient.

Summary

In a study of the staphylococcal carrier state, 4830 cultures were taken in almost equal numbers from the noses and the throats of 222 patients during a 5½-month period of observation on a hospital ward. The mean per cent of nasal cultures containing coagulase-positive staphylococci was 19.2 while, in throat cultures, the corresponding rate was 7.5 per cent.

Coagulase-negative staphylococci were found in 73.2 per cent of nose cultures and in 17.7 per cent of throat cultures.

Carrier rates for coagulase positive staphylococci were found to increase slightly in nose cultures if the period of observation was extended to 60 hospital days. This observation applied to cultures from both antimicrobial-treated and untreated patients. A similar slight increase in nose and throat

carrier rates and in the per cent of patient carriers was observed when cultures taken near discharge were compared to those taken on admission.

Among untreated patients observed for only 30 days there was no increase in rates while, among patients observed for 12 days during treatment with tetracyclines or penicillin, a slight decrease in carrier rates was observed.

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STAPHYLOCOCCAL BACTEREMIA*

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Staphylococcal bacteremia, as a term descriptive of a disease state, is an anachronism. It is realized that reports concerned with such distinctive aspects as endocarditis, in which bacteremia is the rule, appear with regularity.[1, 2] The title "Staphylococcal Bacteremia," however, as a point of departure for a discussion of a sizeable and diversified collection of staphylococcal infections with bacteremia, has been obsolescent for 15 years.

That this is so is doubtless due, in large measure, to the relative success of antimicrobial agents against the *Staphylococcus*, a success that has almost certainly brought about an absolute reduction in the incidence of infections that progress to become bacteremic. Such infections as staphylococcal cellulitis of the face, which formerly were difficult to control and commonly progressed to septic phlebitis and bacteremia, are now usually aborted. Moreover, those cases of staphylococcal disease associated with bacteremia that do occur are now usually reported in conjunction with studies of antimicrobials and their efficacy.

The near absence today of reports on staphylococcal bacteremia emphasizes, in addition, changes in viewpoint or in manner of approach to the problems of bacteremic infection. Staphylococcal bacteremia is, of course, not a disease entity within itself, although in former years authors commonly approached the subject as if it were.[3] Prior to the advent of successful antibacterial chemotherapy, however, the focus of attention was upon the microorganisms and upon their structural complexities and properties that might form a basis for immunologic or other means of "clearance" from the blood. Within the short period of 15 years prior to 1942, numerous and significant papers concerned with the *Staphylococcus*, and with large series of cases of staphylococcal bacteremia, were published. By way of illustration, some of the more significant of the varied approaches prior to the days of antibacterial chemotherapy were: the extensive monograph by Thayer[4] which dealt with the clinical features of endocarditis; the employment of antisera by Julianelle,[5] among others; the study of bacteriophage as a possible therapeutic agent, by MacNeal[6] and by Longacre, Jern, and Meleney;[7] and the review by Skinner and Keefer[8] of 122 cases of staphylococcal bacteremia, including a summary of the literature up to 1941 dealing with experimental infections in animals and the antigenicity of staphylococcal components. Kleiger and Blair's potent exotoxin[9] isolated from some strains of staphylococci exemplifies the staphylococcal components that might underlie manifestations of clinical disease. This exotoxin, upon injection into animals, led to a specific train of events, and its presence appeared to be correlated with the acute symptomatology observed in some cases of staphylococcal infections as they occurred in young people.

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Bacteremia is important since it expresses, in a pivotal sense, the balance between processes whereby bacteria are fed into the blood stream and the mechanisms that are necessary to bring about their removal or destruction. The latter may be attributable to the host, or to antimicrobial chemotherapy. If it is true that time does indeed run out on the successes of chemotherapy, as a result of the widespread development of strains of staphylococci that are resistant to antimicrobials, a significant attack upon problems of staphylococcal infection may necessitate a return to many paths of investigation in which interest has waned with the appearance of antimicrobial drugs—such as inquiries into the nature of bacteria and their attributes and, especially, an intensified study of the host-parasite relationship. Also, prevention of infection at the outset through application of epidemiologic findings[10] may prove to be the important point of attack within institutions that harbor antibiotic-resistant strains and a high incidence of staphylococcal disease.

During the 2-year period from July 1953 to July 1955, at the diagnostic bacteriology laboratory of the Memorial Center for Cancer and Allied Diseases, New York, N. Y., *Staphylococcus aureus* (hemolytic, mannitol-positive, and coagulase-positive) was isolated from the blood, or heart's blood at autopsy, 117 times from 73 patients. This figure is the number found out of approximately 3,000 specimens of blood submitted for culture, 345 of which grew microorganisms of one or another type, including the *Staphylococcus aureus*. The number of cases of staphylococcemia appears to be uncommonly large if comparison is made with nearby general hospitals of comparable size. The strains of *Staphylococcus* isolated from the bacteremic cases fall in a narrow band of bacteriophage types and are frequently drug-fast. This has also been the experience in other clinics.

The patient material seen in Memorial Center, devoted primarily to the diagnosis and treatment of neoplastic diseases, is often of a sort in which, by circumstance, certain facets of the general problem of infection, and specifically staphylococcal disease, are strikingly exemplified. Thus patients with leukemia and other varieties of medical neoplasia, or those who are being treated with irradiation or antitumor chemotherapeutic agents, may have marked disturbances of their reticuloendothelial system, or of other attributes, cellular or humoral, that contribute to the host's capacity to resist infection. Then too, patients who are amenable to treatment by cancer surgery often fall in the older age groups, and they may be considerably depleted biochemically or nutritionally as a consequence of chronic disease, or from cardiac or renal impairment. In this group, wide-sweeping surgical procedures or erosive neoplasms cut across many natural barriers to infection.

Thus the frequency of isolation of *Staphylococcus* from the blood, along with the recognition of the variously depleted capacities for host resistance and our curiosity as to the mode of access of the microorganisms into the blood have prompted a review of such cases.

Materials and Methods

The clinical records of all cases of *Staphylococcus aureus* bacteremia that occurred between July 1953 and July 1955, with the exception of a few in which

the reports of post-mortem findings are still incomplete, were critically reviewed and assessed. The material falls into 4 broad categories in which the staphylococcal bacteremia occurred in patients with (1) cancer, of types considered suitable for surgical treatment and had therefore been so treated, either recently, or in the past; (2) acute leukemia; (3) malignant lymphomas; and (4) a variety of advanced metastatic cancers unsuitable for surgical attack and no longer responsive to therapy directed against the neoplasia. Discussion of groups 3 and 4 has been omitted from this paper.

Some of the cases in group 1, the surgical group, were discarded for want of adequate cultural evidences to authenticate the case. This is particularly true of those cases in which the only isolation of *Staphylococcus* was in mixed culture of heart's blood at autopsy. In all, 16 cases in the surgical category have been selected as unquestionably demonstrating staphylococcal disease associated with bacteremia. Clearly, this arbitrary selection, as well as the complication of neoplastic disease, permits no mortality comparison with other reports.

On the other hand, all cases in group 2, patients with acute leukemia, have been included because alertness to and documentation of the infectious component throughout the course of frequent, often weekly, clinic visits and during hospitalizations while undergoing treatment for leukemia has permitted detailed analysis. In this group there are 23 instances of staphylococcemia.

Analysis of Case Material

Group 1: Sixteen cases of staphylococcal bacteremia arising in patients treated surgically for cancer. Twelve of these patients were more than 60 years of age. Problems of nutritional, cardiac, and renal impairment were common. The veins were frequently in poor condition because of age or previous manipulations. The surgical procedures themselves varied from relatively minor ones, such as incision and drainage of an abscess, to very complicated ones. In several cases the operative procedures were attempts to remedy anatomic defects that persisted after earlier cancer operations. Cancer was not necessarily residual. The bacteremia was directly associated with the immediate surgical procedure in some instances but, in others as, for example, one wherein a staphylococcal perinephric abscess resulted in bacteremia 2 months after nephrostomies were established for the relief of ureteral obstructions due to metastatic cancer, the relationship to surgery was distant or indirect.

While all 16 cases were so chosen as to be indisputable instances of staphylococcal bacteremia, the question of the actual significance of this bacteremia in the total clinical setting did arise. The seriously high mortality rate universally accepted with this disease manifestation is fully appreciated. It was possible, however, that these cases could have been merely expressions of terminal pneumonias in elderly and sorely depleted individuals dying of the many dysfunctions brought about by their chronic neoplastic disease. To clarify this possibility an estimate was made of the relative importance of the cancer and of the infectious component. Three categories were established: (1) cancer hopelessly advanced; (2) cancer and infection both of significance; and (3) sepsis that was found to be of paramount importance or to be present

TABLE 1

RELATIVE CLINICAL SIGNIFICANCE OF CANCER AND INFECTION, COUPLED WITH A TABULATION OF THE FEATURES RETROSPECTIVELY CONSIDERED TO BE OF DOMINANT IMPORTANCE DURING THE BACTEREMIC PHASE. SIXTEEN CASES OF SURGICALLY-TREATED CANCER IN WHICH A COMPLICATING STAPHYLOCOCCEMIA APPEARED

	Cancer hopelessly advanced	Cancer and infection both significant	Sepsis foremost or present alone
Number of patients in each group	4	3	9
Progression of malignant disease	3	0	0
Endocarditis	1	1	5
Meningitis	0	1	3
Cellulitis and/or phlebitis (at sites of intravenous cannulations or absorbable gelatin-sponge implantation)	4	2	7
Deep abscesses	1	1	0
Enteritis	0	0	1
Pyelonephritis	0	0	1
Suppurative pneumonia	0	2	2

alone. At the same time, the features considered to be of dominant importance during the bacteremic phase were estimated retrospectively after a review of all available information (TABLE 1).

In the first category there were 4 patients whose cancer was so far advanced that the bacteremia could be accepted as merely one more factor contributory to an inevitable and immediately impending death. In one of these cases staphylococcal endocarditis was present which, by way of defining the nature of cases included in this category, might well have brought death more slowly than did the encroaching cancer.

There were 3 cases in the second category and, in them, infection and cancer were quite interdependent and jointly significant. One such case was of suppurative pneumonia distal to a bronchial neoplasm that was considered unresectable. It is possible that, in these 3 cases, adequate control of the infectious component might have vouchsafed several additional weeks or months of life to the patient.

In the third group, with 9 patients, staphylococcal sepsis was the major disease and, in several patients, was the *only* significant disease. This provocative finding, along with the high frequency of endocarditis, with its attendant poor prognosis, emphasized the need to determine, if possible, why a complicating infection should arise and how it might be avoided.

To this end, the portals of entry for the *Staphylococcus* that seemed reasonable were itemized after clinical appraisal (TABLE 2). In addition to those customarily in evidence, there was one portal designated "cellulitis at the site of intravascular cannulation." This merits emphasis. Such cannulation was listed more than 30 times, and this figure refers to such procedures only if they were specifically recorded. Not all "cut-downs" are so recorded and, by indirection, many more are known to have been present. Some patients are known to have had 3, 4, or more intravenous cannulations during their hospitalizations, while perhaps only 2 are described in their records. Moreover,

TABLE 2

PLAUSIBLE PORTALS OF ENTRY OF *STAPHYLOCOCCUS AUREUS* INTO THE BLOOD IN 39 INSTANCES OF BACTEREMIA OCCURRING IN PATIENTS WITH CANCER

Portals of entry	Frequency of occurrence	
	Patients with cancer treated surgically (16 cases)	Patients with acute leukemia (22 cases*)
Skin (furuncles, decubitus ulcers, infected herpes)	2	5
Cellulitis at site of finger puncture or bone marrow aspiration	0	12†
Cellulitis and phlebitis at site of intravenous cannulation	>30†	1
Surgical wounds, abscesses	5	0
Gastrointestinal tract	1	2
Nasopharynx (nasopharyngitis with cultures positive for *Staphylococcus aureus*)	0	14
Lungs (pneumonia possibly due to *Staphylococcus*. Clinical or X-ray diagnosis with staphylococci predominant in sputum or throat culture)	7	7
None discerned	0	7

* One patient manifested staphylococcal bacteremia on 2 occasions, separated by a 1 year interval.
† Six cultures taken from site of cellulitis in surgical group, 3 cultures from site of cellulitis in leukemia group. In all instances the staphylococci cultured were of similar bacteriophage types and antimicrobial sensitivities to those organisms isolated from blood.

the tabulation is not that of uncomplicated "cut-downs" but of those in which phlebitis, or sepsis at the point of insertion, necessitated discontinuation. Cultures of the wound, or of the withdrawn catheter, were taken in only 7 of the cases. In these cultures, strains of *Staphylococcus aureus* with typing characteristics similar to those isolated from the patient's blood were demonstrated.

Two instances were noted in which absorbable gelatin sponges might have carried microorganisms and might thus have been responsible for the bacteremia. The operative procedures were for cancer of the side of the face or ear. Extensive surgery around the temporal area resulted in entrance into various venous sinuses, and the sites were then packed with the gelatin sponges. In 1 case bacteremia was associated with purulent meningitis and cavernous sinus thrombosis. Death occurred 2 weeks following the operation. In the other case, although focal acute purulent meningitis was demonstrated at autopsy, the relationship of events seemed to point more to intravenous cannulations than to the meninges as the point of origin for the bacteremia. Cultures taken from an infected "cut-down" site in this instance were positive for *Staphylococcus aureus*. The patient died of staphylococcal endocarditis, with widespread abscess formation, 6 weeks after the operation.

Of the 16 cases, only 2 survived to be completely free of the infectious complication, this despite the fact that in 9 cases infection was uppermost, and in some it was the only disease process. It is noteworthy that the 2 survivors benefitted from unusually well-directed and diligent efforts to diagnose and treat promptly this infectious complication, and that treatment was massive and continued for weeks into the convalescent period.

Group 2: Twenty three instances of staphylococcemia in association with acute leukemia. A review of 23 instances of staphylococcal bacteremia in 22 cases

of acute leukemia led to some unexpected findings, one of the more striking being that 8 patients survived the infection. Other salient features of these cases follow:

In age, the patients ranged from 2 to 33 years. Most, however, were children: 8 were under 5 years of age; 9 were between 5 and 10 years of age; and 4 were between 10 and 15 years of age.

The initial isolation of staphylococci from the blood postdated the first clinical evidence of leukemia for periods that ranged from a month to 2 years. Five patients became bacteremic within the first month after the diagnosis of cancer was established, but only 2 additional instances occurred during the remainder of the first half year. There followed an upsurge, with 6 cases during the period between the 6th and 9th months, and then a lull, with 2 cases between the 9th and 12th months. After that time the frequency increased, with 8 cases.

The duration of life subsequent to the initial detection of staphylococcemia was less than 2 weeks in 13 patients. Two survived more than a month, and were culturally and clinically free of staphylococcal disease at the time of death from hemorrhage secondary to leukemia. Six patients, on the other hand, lived longer than 8 weeks and were free of all evidences of acute staphylococcal infection and bacteremia. One patient recovered from an acute infection associated with staphylococcemia, only to succumb a year later to a second bacteremic staphylococcal infection.

The frequency of dominant clinical manifestations in these cases of staphylococcemia is shown in TABLE 3. Findings that could be attributed equally well to leukemia have been excluded. Six patients displayed extreme toxicity. Erythematous or erythema multiformelike rashes were observed in 5 instances. Respiratory disease was frequent.

Portals of entry considered reasonable in these cases of staphylococcemia are shown in TABLE 2. Election of a probable point of entry was distinctly difficult in this group of patients. In 7 instances none could be discerned. As a rule, staphylococci were carried in the nose, and nasopharyngitis was common. With no other customary point of invasion to be discerned, the site of

TABLE 3

FREQUENCY OF CLINICAL MANIFESTATIONS OBSERVED IN 23 INSTANCES OF STAPHYLOCOCCEMIA IN PATIENTS WITH ACUTE LEUKEMIA*

Manifestations	Frequency
Remittent fever > 104°F.	22
Fever only	7
Extreme toxicity	6
Rash (erythema multiformelike lesions)	5
Cellulitis, phlebitis (at site of skin punctures)	13
Nasopharyngitis (staphylococci present)	14
Pneumonia (staphylococci predominant)	7
Diarrhea (staphylococci in abundance in stools)	2
Miscellaneous	†

* Excluding those clearly attributable to leukemia.

† Jaundice observed in 4 instances, petechiae in most. Such manifestations among others may be ascribable to either disease, but are more likely due to leukemia.

entry was considered to be, in all probability, the nasopharynx. Cellulitis at the site of venepuncture, or of finger puncture, was observed 12 times in close relationship to the bacteremic episode. In only 3 of these instances was a bacteriologic culture of the wound performed.

Analysis of morphology of the bone marrow and of the peripheral polymorphonuclear leukocyte counts for extended periods of time both before, during, and, where possible, subsequent to the initial isolation of staphylococci from the blood, brought out several points. First, there had been nearly total replacement of the normal marrow constituents by leukemic cells in all 23 cases for at least a month prior to the bacteremic episode. Second, peripheral polymorphonuclear leukocyte counts had been repeatedly at a level below 1000 cells cu. mm. for at least a month in 18 instances. This was the case even in 4 of 8 patients who survived and, possibly, in 2 more instances in which observations were unavailable prior to the bacteremia. The third point to emerge was that, in 7 out of 8 patients who managed to survive their staphylococcal bacteremia, a remission of their leukemia, as demonstrated clinically by return toward normal of the bone marrow, peripheral blood picture, and diminution of hepatosplenomegaly, was still possible. Ten nonsurvivors either died too soon for such an effect to be noted or, if they did have examinations, no improvement was evident except in one instance.

The role that ACTH, cortisone, and its analogues might play was similarly considered because these agents were given to most patients, survivors and nonsurvivors alike. No certain estimate could be made as to whether these hormones might have had a deleterious influence, perhaps even to have unleashed the infection. On the other hand, the evidence pointed convincingly in many cases to their beneficial effect toward inducing rapid and effective remission from cancer.

Assessment of the benefits to be ascribed to specific antimicrobial agents must be guarded. One point is certain: no one drug, even in large dosages, and despite *in vitro* evidences of activity, was a panacea. It is equally certain that antimicrobial agents, employed with total inappropriateness of choice, brought no benefit. Between these poles it was noted that staphylococcal bacteremia was frequently first demonstrated in the course of a febrile episode for which the patient was being treated with substantial dosages of tetracyclines. Tetracyclines are commonly prescribed in the pediatric clinics of the Memorial Center for Cancer and Allied Diseases if intercurrent respiratory infections appear to be starting. If the illness progresses, however, and hospital admission is necessary, bacteriologic examinations together with tests for antimicrobial sensitivity are more often used as a guide, or the clinician, fearful of staphylococcal bacteremia while awaiting cultural findings, tends to initiate treatment with chloramphenicol and erythromycin, the agents most often showing successful *in vitro* activity against the staphylococcal strains of this hospital.

As stated earlier, there were 8 survivors in 23 instances of staphylococcemia. In 7 of these 8 survivors bacteremia was initially detected while antimicrobial drugs that were inappropriate by *in vitro* standards (for example, tetracyclines or penicillin), were being employed. Upon receipt of cultural findings, change-

over to treatment with chloramphenicol and erythromycin usually followed. The high recovery rate, while upon presumably adequate chemotherapy, suggests that appropriate antimicrobial agents, even when delivered late, are of the foremost importance. To emphasize the difficulty of assessment in so multifactorial a setting, it must be pointed out, however, that there were an additional 8 patients who, instead of receiving tetracyclines, were being carried fortuitously upon treatment from the outset and throughout with other agents that would be regarded as appropriate on the basis of *in vitro* sensitivity tests. Contrary to our expectations, only one of these patients survived. The failures could not be attributed to the development of drug-resistant strains, nor were differences detected in other specific variables alluded to thus far.

The course of the single patient just mentioned illustrates many features of the type of patient material — the cases of acute leukemia — under discussion, and is presented below (FIGURE 1):

"The patient, a ten year old girl (M. H. # 25051), was diagnosed to have acute leukemia 13 months before the complication of importance to this discussion, staphylococcemia, was detected. For 8 months initially she had been managed with anti-leukemic chemotherapy successfully, but, over a period of five months prior to the initial isolation (March 2, 1954) of staphylococcus from the blood, she became increasingly refractory to these agents. Many examinations of the bone marrow smears of this child showed it to be near complete replacement with 'stem' cells. The concurrent peripheral white blood-cell counts were often less than 1000 granulocytes per cu. mm.

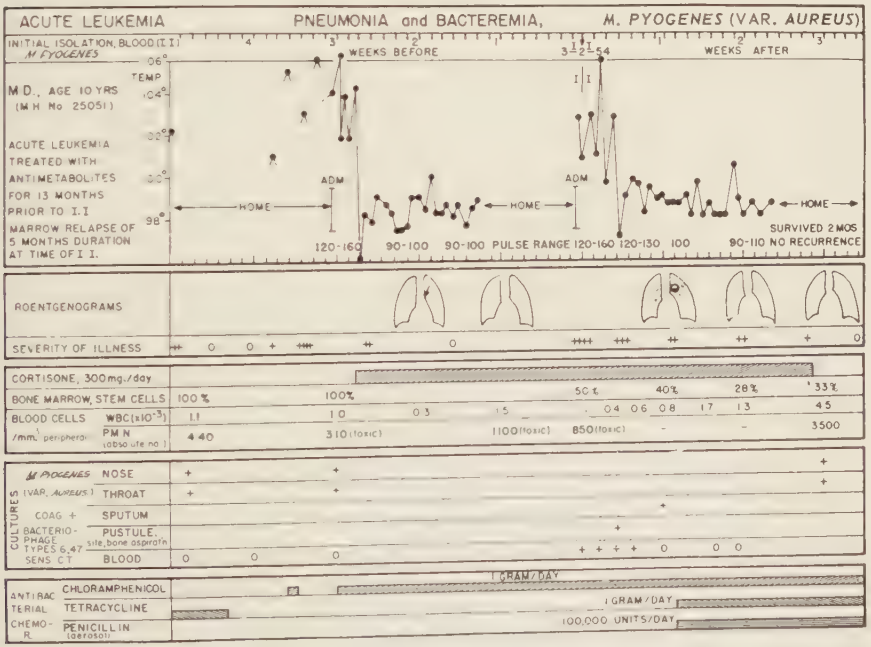

FIGURE 1. Staphylococcal pneumonia and bacteremia in a patient with acute leukemia.

"Four weeks before the primary isolation of *Staphylococcus* from her blood, the patient had an acute respiratory illness with radiographic evidence of bronchopneumonia. Chloramphenicol therapy was begun, together with cortisone which was used in the hope of inducing rapid remission of the cancer. Both these therapeutic agents were continued for a prolonged period.

"Clinical improvement was shortly apparent, and the pulmonary infiltrations were largely cleared within ten days. Although convalescence seemed well established, and while continuing the same therapeutic regimen, she suffered an abrupt clinical relapse. She again entered the hospital, seriously ill, septic and with high fever.

"Blood cultures drawn on entry and on three subsequent days were positive for *Staphylococcus aureus*. Her sputum, as well as pustules at the site of a bone marrow aspiration, yielded staphylococci with characteristics identical to those previously present in the nasopharynx, as gauged by similarity of pattern to bacteriophage typing and antimicrobial resistance. The chest roentgenograms again disclosed bronchopneumonia, more extensive than previously. There were in addition several thin-walled, ring-like areas which were considered to be infected bullae. Without change in antimicrobial or hormone therapy, the fever remitted, pulmonary signs again began to clear, and the clinical illness subsided. Staphylococci were not again cultured from the blood. Such evidences of improvement were all obvious prior to adding tetracycline, and penicillin aerosol, to her regimen.

"During the sequence of events described, and for months before, the peripheral granulocyte count was remarkably low, although the trend toward bone marrow remission became evident as the bacteremic illness was running its course, and was fully apparent 3 weeks after the staphylococcemia became manifest. She survived two months without recurrence of signs of infection. Death was due to hemorrhage, secondary to the leukemia."

Discussion

There has always been agreement upon the serious outlook in cases of bacteremia due to *Staphylococcus aureus*, and this is still so even though chemotherapeutic agents have had a moderate success in the established case. Chemotherapy may even have lowered the over-all incidence of such cases by successfully aborting infections that, when unchecked by antimicrobials, carry an appreciable likelihood of becoming bacteremic. Nevertheless, the presence of an unexpectedly high incidence of bacteremic cases within institutional areas, along with the predominance of strains that are drug-fast, impels a close scrutiny as to why this should be so. It is clear that an understanding of the factors that may lead to the bacteremia is of prime importance and is basic to its successful control.

The survey of cases within our own institutional boundaries has brought forth similarities to the experience of others, as well as dissimilarities. In the former category, there is present within the hospital, both in personnel and in long-term patients, an appreciable carrier rate of staphylococci that are predominantly drug resistant (by *in vitro* testing) to penicillin, streptomycin, and the tetracyclines, although they remain susceptible in general to erythromycin

and chloramphenicol. The majority of strains fall, in addition, into a limited range of bacteriophage types, the chief ones being 6, 7, 47, 44a, and 42e. The staphylococci that were isolated from these bacteremic cases are nearly uniform in their basic characterizations. Moreover, they differ substantially in antibiotic pattern, frequency of bacteriophage typability, and range of types, from staphylococci that were isolated in the external environment in the course of an epidemiologic study carried out in these laboratories.[11] All of this points toward a high incidence of staphylococcal strains that are closely similar, and suggests that infection with such strains may be acquired within the hospital environment.

Points of dissimilarity in our cases appear to reflect largely an unusual distribution of host types within the institution. These individuals, for reasons of age or because of neoplastic disease, may be depleted in powers of resistance to infection, or may run a higher risk of invasion by microorganisms, from the application of therapeutic modalities that are of great convenience or are necessary in these patients but less commonly used among general hospital patients.

Analysis of the portals of entry has led to an unexpected surmise, namely, that sustained intravascular introduction of the staphylococci may be of major importance in accounting for the frequent occurrence of bacteremia in the surgical group of cases. The evidence for this surmise is very suggestive and is quite worthy of further study and action. Attention was drawn to cellulitis at the site of intravenous cannulations that are resorted to with particular frequency in the surgical cases in pursuit of diagnostic or therapeutic measures. In the 7 instances where culture of the site of cannulation was performed, culture of the blood was unfortunately not done at the same time. Isolation from the blood, of staphylococci of similar typing characteristics, at another time, however, permits an inference of the association of septic cannulation with bacteremia, or of a cause and effect relationship, that is difficult to overlook. Although direct venous introduction is not considered a common mode of entry for microorganisms, it has been observed, especially in "main line" narcotic addicts. There are previous reports in which staphylococcal endocarditis has been attributed to such cannulation as, for instance, in the case described by Herrell, Nichols, and Martin,[12] of a patient with poliomyelitis who required repeated intravenous cannulations.

Septic thrombophlebitis, if it occurs in conjunction with staphylococcal cellulitis at the site of intravenous cannulation, may be responsible for continued bacterial embolization, even after discontinuation of the cannulation. Lyons,[13] 15 years ago, in a discussion of surgical staphylococcal infections, re-emphasized similar mechanisms of pathogenesis that have long been recognized and are basically the same in principle.

There have been previous demonstrations, too, of the potential dangers of absorbable sponges when implanted, after impregnation with bacteria, into experimental animals.[14] Inadvertently contaminated sponges which served as the underlying focus of bacteremia in human beings have also been reported.[15]

Some comment is warranted as to the frequency of cannulations that we have recorded, whether they are excessive, or in what way our experience with

them may differ from that of others. The answer lies largely in the number and nature of medical and surgical problems dealt with in this hospital, where an impressive number of surgical procedures are performed daily in the effort to control cancer. This makes inevitable the inclusion of many patients who require extensive, extirpative surgery. Nutritional, electrolyte, and other therapeutic controls are often realistic only when intravenous cannulation is effected.

If the premise is correct that cannulation serves as a portal of entry, and that bacterial cellulitis at the site contributes the microorganisms, then cannulation should be avoided unless absolutely necessary. Cellulitis at the site of cannulation should alert the physician not only to the need for removal of the device but also to the need for bacteriologic culture of the wound and of the impending possibility of persistent bacteremia.

A review of the surgical cases in particular made it clear that staphylococcal bacteremia does not always present itself in a dramatic manner. Because of this there is frequently a delay in applying bacteriologic techniques necessary to the diagnosis. Moreover, the serious implications of staphylococcal bacteremia are commonly underestimated, and the necessity for prolonged, intensive, and appropriate chemotherapy is often not appreciated. Stereotyped employment of prophylactic antimicrobials promotes, too, a sense of security that may be quite unwarranted in that infectious components, if present, may remain untouched or be masked by the therapy. Meanwhile, alertness lags.

Even though the diagnosis were to be established early, however, and though therapy were to be ideally managed, the fact would still remain that emphasis should be placed, in so far as possible, on avoidance of factors that favor sustained staphylococcal bacteremia. This would materially reduce the incidence of endocarditis and would save more lives than the most impressive treatment of established cases.

In cases of acute leukemia, staphylococcal cellulitis at the site of finger puncture was observed. Whether this represents focalization of staphylococci at such a point of trauma secondary to a bacteremia that is arising from some other portal, for instance, from the nasopharynx, is not known. It does appear possible, however, that small children, who are in most instances nasal carriers of staphylococci, infect these puncture wounds from their own noses.

Turning to other aspects of infectious processes as seen in association with acute leukemia, in particular to bacteremic staphylococcal infections, some findings of unusual interest emerge. The conviction appears widespread among clinicians that patients with this neoplastic disease are especially vulnerable to infections, a viewpoint that may arise from the frequent observation of overwhelming bacterial sepsis when death is imminent from leukemia. In a patient with acute leukemia there are many derangements that might be expected to enhance susceptibility to infection or to impair the host's powers of resistance. Thus cancerous replacement by primitive leukocytes of the normal structure of the bone marrow, which becomes evidenced by anemia, thrombocytopenia, and entry into the peripheral circulation of granulocytes defective in both type or number, would lead one to suppose that if the integrity of the marrow is representative in part of the functional adequacy of host defense defense

might then be poor indeed. Extensive cancerous infiltration of the liver, spleen, and, to some extent, the lymph nodes, might reasonably be assumed to affect, directly or indirectly, the fixed macrophages of the reticuloendothelial system, as well as the production of antibodies.[16] Adrenocortical hormones are commonly employed in treatment. These have repeatedly been shown to alter, usually for the worse, host reaction to infection. Finally, in the course of acute leukemia and its treatment, ulcerations of the mucosal linings often occur, thereby breaking one more normal barrier to invasion.

The rapidity of progression in acute leukemia is such that, if untreated, death usually occurs within a few months after its clinical recognition.[17] Present-day therapy, with folic acid antagonists, purine analogues, adrenocortical hormones, supportive blood replacement, and antimicrobial therapy, extends the median life expectancy in children with acute leukemia to a year or longer.[15] As long as antileukemic therapy is effective, remission of the cancer may be so complete that it is impossible to detect essential deviations from the normal by either clinical or laboratory studies. By implication then, the susceptibility to infection is conceivably that of the normal individual, so that, with good management, there would seem to be no *a priori* reason to expect undue susceptibility to infection during phases of complete remission.

This is not a matter of black and white, however. Shades of gray are easily detected during periods of progression or regression of the cancer. It is during these phases, particularly, which sometimes last for months, that factors appear to be operative which, in an unknown manner, afford more protection for the host against invading microorganisms than would be anticipated. While it was observed that the cases of staphylococcemia occurred with a somewhat periodic frequency that was suggestively related to periods of leukemic progression, extended intervals were strikingly in evidence during which the bone marrow and peripheral blood were grossly deranged and hepatosplenomegaly was marked, without the presence of infection.

Throughout their entire course, most of these patients carried abundant staphylococci within their noses or throats and, in general, went through several episodes of cancer relapse without bacteremic invasion by this microorganism. This is evident from the fact that 15 patients lived a year, and through one or more relapses of their cancer before the staphylococcemia was manifest. In these patients there were the usual intercurrent, upper-respiratory infections, and the acute infectious diseases of childhood, but scant evidence of serious bacterial disease.

A point was made earlier of the unexpectedly high rate of survival of these patients with acute leukemia and bacteremic staphylococcal infection. Whether this is conceivably related to diminished virulence of the strains, with attendant alterations in such attributes as invasive potential or toxin production, we do not know. Recovery from the infection appeared to parallel the demonstration that cancer regression could still result from treatment with antitumor agents.

In the experimental study of infectious diseases, use is made of animals that have been variously altered for purposes of analyzing the host factors concerned in infection. In the group of patients with acute leukemia who have been

presented here and, also, in some other neoplastic diseases such as the malignant lymphomas, nature has provided a ready-made experimental system in which much may be learned of the attributes of the human host whose derangements may frequently be comparable to such animal preparations.

For more than a decade attention on the antimicrobial approach to infectious diseases has been intense. The current renaissance of interest in the properties of the host especially, and of the invading microorganisms, promises to be fruitful, not only theoretically, but in the practical conquest of the problems of infection that remain.

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MICROCOCCIC ENTERITIS AND PSEUDOMEMBRANOUS ENTEROCOLITIS AS COMPLICATIONS OF ANTIBIOTIC THERAPY

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The purpose of this paper is to present studies on a series of patients in whom resistant strains of coagulase-positive *Micrococcus pyogenes* (*Staphylococcus aureus*) appeared in the intestinal tract in varying numbers after the oral administration of various antibiotic agents that markedly diminished or eliminated the normal intestinal bacteria.

These studies could be presented in many different ways, but perhaps it would be well to review the circumstances that initiated the investigations at our institution into the problem and then to record some of the clinical and laboratory data collected on patients with micrococcic enteritis and pseudomembranous enterocolitis.

Background Data

In 1952 and early in 1953, during the course of a planned study to learn more about pseudomembranous enterocolitis and postantibiotic diarrhea, it was found that the stools in some of the latter group of patients contained *M. pyogenes* in pure culture.

A review of the literature at that time indicated that others had previously recorded findings pertinent to this problem. Jackson and his associates,[1] stated that 7 out of 91 patients with pneumococcal pneumonia died after administration of Terramycin. In 3 of the 7 fatal cases severe diarrhea was present, and cultures of both the sputum and the stools disclosed *M. pyogenes*. Janbon and his co-workers[2, 3] reported severe diarrhea in 9 of 200 patients who had various types of disease and who received Terramycin. They considered staphylococci to be important in this complication and advised that diarrhea was an indication to discontinue administration of the drug and to institute symptomatic therapy. Reiner and his colleagues[4] reported necropsy studies in 5 cases of pseudomembranous colitis after use of Aureomycin and chloramphenicol. Cultures of the stools did not show any enteric pathogens. These investigators considered the antibiotic agent to be the cause of pseudomembranous colitis. Haight and Finland[5] reported 1 case of pneumococcal pneumonia treated with Terramycin in which diarrhea developed and in which *M. pyogenes* was found in cultures of the stools. Recovery occurred after the administration of erythromycin. Bernhart[6] recorded 2 cases of fatal enterocolitis associated with the use of Terramycin. In 1 of these cases, micrococci were found in the diarrheal stools. Meier[7] reported 3 cases of fatal circulatory collapse after the use of Terramycin. He assumed that staphylococci were the cause. Heilman and I[8] reported our initial experience with micrococcic enteritis in March 1953. Since that time numerous publications have appeared

dealing with various aspects of the problem, among which are the papers of Terplan and his co-workers[9] and Speare.[10]

The bacteriologic methods employed in the present study are the same as those described in a previous publication.[8] The pathologic criteria for pseudomembranous enteritis or enterocolitis are the same as those recorded by Pettet and his associates.[11]

There has been considerable confusion in the literature regarding the significance of postantibiotic diarrhea. This diarrhea may occur without culturable *M. pyogenes* in the stools. A series of 15 patients has been observed with post-tetracycline diarrhea that disappeared without any specific treatment. Discontinuation of the use of the oral antibiotic was all that was required to permit the diarrhea to cease within a few days. Culturable *M. pyogenes* was not found in the stools of these patients at any time during the course of the diarrhea. Negative stool cultures, along with various combinations of yeasts, *Proteus*, and *Pseudomonas*, were the rule in these patients, but a few of the patients also had the usual flora present in the intestine. In several of the individuals who underwent proctoscopic examination and roentgenographic study of the colon there was no evidence of intestinal inflammation. The cause of the diarrhea in this group of patients was not determined by controlled studies, but it can be stated that *M. pyogenes* was not cultured from the intestinal contents during the stage of active diarrhea.

On the contrary, there is no reasonable doubt that *M. pyogenes* growing in large numbers and in more or less pure culture in the intestinal tract of man can give rise to definite gastrointestinal and systemic complaints. These symptoms vary in intensity from simple weakness, nausea, mild abdominal distention, low-grade fever, and a few loose stools to severe exhaustion, marked nausea, severe vomiting, extreme abdominal distention, high fever, excessive sweating, copious diarrhea, mental confusion and, finally, mild degrees of shock.

Present Series of Cases

Studies designed to show the relationship between the gastrointestinal as well as the systemic symptoms and the growth of *M. pyogenes* in the intestinal tract, both with and without pseudomembranous enterocolitis, are grouped, for the purpose of simplifying the presentation, as follows:

Group 1 (20 cases).* This group includes those patients in whom intestinal cultures revealed either a few micrococci (*M. pyogenes*) along with many colon organisms (*Escherichia coli*) and other normal intestinal flora, or moderate numbers of micrococci associated with the usual intestinal bacteria. The 20 patients with only a few micrococci in the intestine did not exhibit any untoward gastrointestinal complaints while, in the 6 patients with moderate numbers of micrococci, mild diarrhea, slight abdominal distention, and some systemic weakness did develop. Discontinuation of the use of the oral preparation of tetracycline was sufficient to permit the normal intestinal flora to suppress the growth of *M. pyogenes*. The mild symptoms disappeared as these micrococci decreased in numbers in the intestinal tract.

* Six cases reported previously as cases 1 to 6.[8]

This group of patients illustrates that a few resistant micrococci may exist in the intestine without giving rise to any symptoms and that, even though moderate numbers of micrococci exist in the intestine, the symptoms may be mild to moderate, yet temporary, as long as the usual bacterial flora is present in the intestine and use of the tetracycline derivatives, if they are being administered, is discontinued.

Group 2 (42 cases).* This group is composed of those patients in whom varying degrees of nausea, vomiting, diarrhea, abdominal distention, fever, weakness, and exhaustion developed and who simultaneously had in the intestinal tract large numbers of *M. pyogenes* in more or less pure culture. The usual intestinal bacterial flora was absent or present in relatively small numbers. The patients in this group received one of the tetracycline derivatives for various types of diseases or for preparation of the intestine for surgery. It was among this group of patients that we first observed that *M. pyogenes* was a factor in some of the cases of postantibiotic diarrhea. Each patient in this group received, by mouth, erythromycin (300 to 400 mg. 4 times a day) or, less commonly, bacitracin (20,000 units 4 times a day), or neomycin (1 gm. 4 times a day). All of the untoward symptoms disappeared as the micrococci were eliminated from the intestinal tract. Three patients in this series were given novobiocin orally (500 mg. 4 times a day). This antibiotic removed the intestinal micrococci, and the untoward symptoms disappeared.

This relatively large series of patients illustrates that significant numbers of resistant strains of *M. pyogenes* are not uncommon in the hospitalized patient when one of the tetracycline drugs is administered orally. It also shows that micrococcic enteritis is a reversible disease and is far from being universally fatal as some authors would lead one to believe.

Group 3 (12 cases†). This group consists of patients who were prepared for intestinal operations with oxytetracycline given orally and were found to have *M. pyogenes* in the intestine in more or less pure culture at the time of the operation. The administration of oxytetracycline was discontinued, and erythromycin was given by mouth immediately postoperatively in doses of 300 to 400 mg. 4 times a day. *M. pyogenes* disappeared from the intestinal tract, and no untoward gastrointestinal or systemic reactions occurred.

This group of patients demonstrates that even though *M. pyogenes* organisms are present in the intestine in pure culture they can be eliminated by the prompt administration of the proper antibiotic agent before the micrococci have had sufficient time to produce significant untoward symptoms.

Group 4 (4 cases‡). This group also includes patients in whom *M. pyogenes* was found in the intestine in pure culture at the time of the operation. Administration of oxytetracycline was discontinued in these patients and they were observed for untoward systemic symptoms related to the presence of micrococci in the intestine. All patients so observed experienced rather severe gastrointestinal and systemic reactions. These untoward symptoms subsided as the micrococci disappeared from the stools after subsequent administration of erythromycin was begun. This group enhances the evidence that *M.*

* Eighteen cases reported previously as cases 20 to 37.[8]
† Nine cases reported previously as cases 7 to 15.[8]
‡ Four cases reported previously as cases 16 to 19.[8]

pyogenes was the etiologic agent in these untoward gastrointestinal and systemic complaints.

Group 5 (11 cases).* This group consists of those patients who received a tetracycline derivative and who experienced nausea, vomiting, distention of the abdomen, severe diarrhea, fever, mental confusion, and shock, and who finally died. Necropsy showed pseudomembranous enteritis or enterocolitis, and cultures of the intestinal contents at the time of death revealed numerous micrococci (*M. pyogenes*) in pure culture. This group illustrates that pseudomembranous enterocolitis and *M. pyogenes* may exist at the same time in the intestine of the patient.

Group 6 (4 cases). This group is composed of those patients in whom necropsy studies showed pseudomembranous enterocolitis and either a few *M. pyogenes* organisms or moderate numbers of *M. pyogenes* along with the usual mixed intestinal flora. These findings indicate that pseudomembranous enterocolitis may exist with the normal intestinal flora plus varying numbers of *M. pyogenes*. It was noted in group 1 that mild micrococcic enteritis might be present with the normal flora plus varying numbers of *M. pyogenes* in the intestine.

Group 7 (5 cases†). In this group are included those patients who died of pseudomembranous enteritis or enterocolitis (confirmed at post-mortem examination), but in whom cultures made from the intestine both before and at the time of death failed to reveal *M. pyogenes* or any enteric pathogens. One of the patients in this group did not receive any antibiotic agent at any time orally or parenterally.

This group illustrates that pseudomembranous enterocolitis may exist in patients without culturable *M. pyogenes* in the intestine. The group is compatible with many of the cases reported by Kleckner and his associates[12] and by Pettet and his associates,[11] except that these investigators did not study thoroughly the bacterial flora at the time of death of the patients and did not use selective media to permit the recognition of *M. pyogenes* if present.

In the near future my colleagues and I will publish the detailed clinical, bacteriologic, and pathologic findings in patients with pseudomembranous enterocolitis.

Group 8 (2 cases‡). This group consists of patients who had typical clinical symptoms of micrococcic enteritis (nausea, vomiting, severe diarrhea, abdominal distention, fever, rapid pulse rate, extreme exhaustion, and mental confusion) and who had large numbers of micrococci (*M. pyogenes*) in pure culture in the intestine at the time of death. The cause of death (confirmed at necropsy) of one of these patients was considered to be peritonitis following separation of the sutures at the site of removal of a sigmoidal polypoid lesion. The other patient underwent resection for an intestinal neoplasm but died postoperatively of acute myocardial infarction (proved at necropsy). Both patients had received antibiotic therapy preoperatively and postoperatively. At necropsy the mucosa of the intestine in each patient failed to show pseudomembranous enterocolitis.

* Four cases reported previously as cases 39 to 42.[8]
† One case reported previously as case 43.[8]
‡ One case reported previously as case 38.[8]

This group is interesting in that there was no evidence of pseudomembranous enteritis or enterocolitis in spite of the clinically severe micrococcic enteritis and positive cultures for *M. pyogenes* in the intestine at the time of death. It illustrates that micrococcic enteritis can exist without the presence of a pseudomembrane in the intestine. This finding is compatible with the clinical impressions entertained in groups 2, 3, 4, and 5.

Group 9 (6 *cases*). In this group the clinical diagnosis of pseudomembranous enterocolitis was made, based on the presence of nausea, vomiting, abdominal distention, severe diarrhea, fever, tachycardia, mental confusion, and definite clinical shock. All of these patients received one of the tetracycline derivatives before the onset of their illness, and use of the drug was discontinued promptly as soon as the diarrhea developed. Three of the patients had *M. pyogenes* in the intestine in pure culture. The administration of erythromycin removed the micrococci from the intestine, while supportive measures were instituted to combat the shock as well as the loss of fluid and electrolyte. These 3 patients recovered completely. The other 3 patients, unfortunately, did not have stool cultures made but recovered after the use of measures designed to combat shock and the loss of fluid and electrolyte. It is not known whether *M. pyogenes* was present or absent in the stools of these 3 patients, but antimicrococcal drugs were not used in their management.

It is granted that the diagnosis of pseudomembranous enterocolitis in these 6 patients is presumptive and based on clinical findings plus severe degrees of shock. In our experience, micrococcic enteritis (uncomplicated by septicemia, peritonitis, pneumonia, and so forth) is seldom accompanied by more than mild degrees of shock, while severe shock is the rule in pseudomembranous enterocolitis with or without the presence of *M. pyogenes* in the intestine.

Discussion

These data indicate that not all postantibiotic diarrhea is related to *M. pyogenes* in the stools but that there is a clearly definable symptom complex related to the presence of large numbers of resistant micrococci in the intestine of patients who have received antibiotic agents that have suppressed the normal intestinal flora.

It was postulated in 1953[5] that the symptoms produced in micrococcic enteritis are the result of toxins produced by *M. pyogenes* growing in large numbers in the intestinal tract. Surgalla and Dack[13] have presented experimental evidence to show that 30 of 33 strains of *M. pyogenes* isolated from the intestinal tract of patients with micrococcic enteritis produced an enterotoxin. It is presumed that the enterotoxin gives rise to the gastrointestinal as well as to the systemic symptoms.

Under normal conditions *M. pyogenes* is seldom present in the feces and never present in large numbers. When the usual bacterial flora of the intestine is inhibited by the administration of antibiotic agents, strains of *M. pyogenes* that are resistant to these antibiotic agents may proliferate rapidly in the intestine and produce both systemic and gastrointestinal symptoms. The micrococci will persist, unless removed by chemotherapy, until they are replaced by the normal intestinal flora.

It was noted in the course of our initial investigation that many of the patients in that study harbored resistant strains of *M. pyogenes* in their throats. This suggests that these antibiotic-resistant organisms are transferred from the upper part of the respiratory tract and pharynx of 1 patient to that of another in the hospital, or from hospital personnel to the patient. If a person harboring these resistant strains of micrococci in the upper respiratory tract or throat receives, for any reason, an antibiotic agent that is capable of suppressing the normal intestinal bacterial flora, these micrococci may pass into the intestinal tract where they will grow in profusion because of the absence or greatly decreased presence of the normal intestinal flora. Patients have been observed who received antibiotic agents that eliminated the intestinal flora completely at the time of an operation on the intestine, but who, a few days later, had antibiotic-resistant *M. pyogenes* in the intestine in large numbers. It is presumed that the micrococci passed into the intestine from the nasopharynx where they were cultured in varying numbers. Thus it cannot be assumed in the hospital that, if the intestine is free from all bacteria at the time of surgical resection for an intestinal lesion (such as carcinoma), micrococci will not appear in the bowel within 3 to 9 days postoperatively to produce micrococcic enteritis.

These studies indicate that when diarrhea develops in a patient during the course of oral or parenteral administration of any antibiotic that may significantly decrease the normal intestinal flora, use of the antibiotic should be discontinued immediately and both smears and cultures should be made from the stools. If the smear reveals micrococci, treatment with some chemotherapeutic agent should be started promptly. Erythromycin is the agent most commonly used at the present time but, if sensitivity studies indicate resistance to erythromycin, then bacitracin, novobiocin, neomycin, or some other appropriate antibiotic should be used. If the patient is not seriously ill and *M. pyogenes* is not present in large numbers, discontinuation of the administration of the antibiotic agent to which micrococci are resistant may be all that is necessary (especially if the patient is able to eat an adequate diet).

These studies show that pseudomembranous enterocolitis may be associated with *M. pyogenes* in pure culture in the intestine or that the pseudomembrane may exist in the intestine without culturable *M. pyogenes*. Furthermore, the studies indicate that *M. pyogenes* may be present in pure culture and may be associated with the characteristic symptoms of micrococcic enteritis when no pseudomembrane can be demonstrated in the intestine. Hence, it is possible that *M. pyogenes* may be a factor in accentuating or producing some of the cases of pseudomembranous enterocolitis. This organism, however, does not offer an adequate basis for explaining all the cases of pseudomembranous enterocolitis presented in these studies.

These findings emphasize a fact that has been stressed by many writers, namely that antibiotic agents should be used only when they are specifically indicated and should not be used promiscuously for the treatment of minor illnesses. These agents, even though they suppress the normal intestinal flora, are not contraindicated in diseases that respond to their use.

Conclusions

Not all patients with postantibiotic diarrhea have culturable *Micrococcus pyogenes* in the intestinal tract.

Antibiotic-resistant strains of *M. pyogenes*, when present in small numbers along with the normal intestinal flora, may not produce any symptoms. On the contrary, these organisms, when present in large numbers or in pure culture in the intestine, may produce varying degrees of fairly characteristic gastrointestinal and systemic reactions that have been called "micrococcic enteritis."

Micrococcic enteritis, produced by *M. pyogenes*, may exist without any definite pseudomembrane in the intestine.

Micrococcic enteritis, even when marked, is a readily reversible disease with proper chemotherapy and supportive measures.

Conversely, pseudomembranous enteritis or enterocolitis is a serious illness and may exist both with and without culturable *M. pyogenes* in the intestine. Hence, this microorganism may be a factor in accentuating or producing pseudomembranous enterocolitis, although it does not offer an adequate basis for explaining all cases of pseudomembranous enterocolitis.

These studies show that it is possible to have a few patients with clinically diagnosed pseudomembranous enterocolitis recover after adequate supportive measures and perhaps antibiotic chemotherapy whenever *M. pyogenes* is also present in the intestine.

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Discussion of the Paper

Leighton E. Cluff (*The Johns Hopkins Hospital, Baltimore, Md.*): Sumner Wood and Ivan Bennett of The Johns Hopkins Hospital have recently studied an outbreak of a peculiar enteritis in a commercial colony of chinchillas.[1] Histologic examination of the gut in animals dying of diarrhea showed classic lesions of pseudomembranous enterocolitis. The pseudomembrane contained huge clumps of gram-positive cocci. Detailed cultural studies were carried out, and it was shown that the disease in chinchillas was associated with an overgrowth of the normal intestinal flora by hemolytic staphylococci. In many ill animals throat and rectal swabs yielded pure cultures of this organism. The colony had been fed for a period of 18 months on pellets containing nutritional amounts of chlortetracycline and, when pellets of similar nutritional composition containing no antibiotics were substituted for this diet, the enteritis stopped and serial cultures showed a gradual disappearance of staphylococci from the stools of the animals. At the present time an attempt is being made to reproduce this disease in the laboratory using the chinchilla as an experimental host. Nothing is known about the incidence of this disease in chinchillas, and this occurrence is reported simply to call attention to what is believed to be the first recognition of staphylococcal enteritis in an animal species and as a promising lead in the experimental study of the relationship between antibiotics, staphylococci, and pseudomembranous enterocolitis.

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THE UNKNOWNS OF STAPHYLOCOCCAL INFECTION

By René Dubos
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The greatest merit of this monograph is the emphasis that it places on all the uncertainties of our knowledge concerning the bacteriology, the pathogenesis, and the epidemiology of staphylococcal infections. Unfortunately, little positive new information will be found in these pages. The only recourse remaining to the text-book writer will be to heed Chesterton's advice and

. . . never, never doubt
What nobody is sure about.

For the investigator, on the other hand, these papers will have mapped out many areas in which critical research is greatly needed. Among the many questions that cry out for an answer, I shall mention but a few of those that are implicitly or explicitly raised in this publication.

Epidemiology of Staphylococcal Infections

Two related questions of epidemiological interest are discussed with conflicting views throughout this publication. One concerns the extent to which staphylococcal infections are becoming more prevalent and severe, either in hospitals or in the general population. The other has to do with recent reports that certain strains of *Staphylococcus* are peculiar in their ability to give rise to epidemics or to pathological states of great severity.

We find in these pages several dramatic testimonials that tend to suggest that the *Staphylococcus* problem is rapidly increasing in practical importance, but these testimonials refer to localized outbreaks rather than to epidemic situations. On the other hand we also find that staphylococcal infections in hospitals can be effectively controlled by the simple application of rigid aseptic techniques, although doubt has also been expressed as to the practicability and lasting efficacy of protective measures under practical conditions. Related to these problems are the many reports that certain medical procedures do increase the susceptibility of man to staphylococci.

It is not likely that an adequate picture of the extent and importance of staphylococcal disease will emerge from the mere recital of individual experiences. To determine the magnitude of the problem and to identify its determinants it will be necessary to conduct surveys according to the proper epidemiological criteria. These epidemiological surveys would naturally acquire more meaning if it were possible to identify the strains of staphylococci normally present in the general population and those strains responsible for particular outbreaks.

Much interest, theoretical as well as practical, has been added to the problem of strain identification by the many reports that cultures can rapidly undergo profound changes in some of the characteristics that affect their behavior *in vivo*. In consequence it would appear necessary that staphylococcal strains be characterized in the form in which they occur in diseased tissues, rather than as laboratory subcultures. These problems of laboratory diagnosis will cer-

tainly demand the development of new techniques and new reagents. In addition to the determination of serological and bacteriophage types and to the measurement of drug resistance, it may prove useful to estimate quantitatively some of the components and products of staphylococci that are assumed to be of significance in pathogenesis. Certain laboratories should become specialized in these diagnostic studies since many of the techniques and reagents involved are not readily available to the hospital technician.

The Persisters

All investigators who have followed the fate of staphylococci *in vivo* have found that small numbers of these organisms persist for prolonged periods of time in various organs even under conditions that appear favorable for their rapid and complete elimination. This situation has been observed, for example, in animals with a clearing mechanism so highly and rapidly effective against staphylococci that the largest percentage of the injected organisms are killed within 2 hours after injection. Equally surprising is the fact that staphylococci persist in human beings or in animals receiving chemotherapy, even though the persisters may remain fully susceptible to the drug in use. Since it can hardly be doubted that these persisters are potentially of great practical importance, additional information is needed concerning the factors that account for their origin and that determine their ultimate behavior in the infected host. A few questions immediately come to mind in this regard.

What is the nature of the local *in vivo* environment, intracellular or extracellular, that provides for staphylococci a shelter that protects them against the host defense mechanisms and antimicrobial drugs?

What are the morphological or physiological characteristics that endow staphylococci with the ability to persist *in vivo*? Are the persisters chiefly present in some peculiar phase of a life cycle, corresponding, for example, to the degraded G or D forms? Or do they consist of more normal cellular forms that exhibit a high degree of resistance to inimical agents merely because they are in a semidormant, resting metabolic state?

What are the circumstances that upset the equilibrium between host tissues and persisters and allow the latter to resume multiplication? It would be important to know what changes in the immune state of the host or what physiological factors can thus be responsible for reactivation of the infectious process.

Determinants of Virulence and Reactions of Immunity

Among pathogenic microorganisms, staphylococci stand in a special position by virtue of the large numbers of their cellular constituents and products that have been claimed to be correlated with virulence. Production of pigments, of coagulase, of hyaluronidase, of leukocidin, of various hemolysins, of phosphatase, and so forth are all cultural characteristics that occur with a very high degree of frequency among virulent strains. On the other hand, it is equally true that none of these characteristics has been convincingly shown, thus far, to play a determinant part in the ability of staphylococci to become established *in vivo* or to cause disease. Fortunately several of the papers in this monograph have revealed interesting new facts that will certainly serve as useful guides for

future studies on the relations that exist between cultural characteristics and pathogenic properties.

It has been repeatedly emphasized, for example, that staphylococci can undergo rapid and profound alterations in some of their most interesting properties. Some of these alterations appear to be hereditary, the results of mutation-like phenomena. They become manifest when some change in the environment, *in vitro* or *in vivo*, gives a selective advantage to the mutant forms.

Cases have also been described in which the phenotypic manifestations of the genotype are markedly influenced by physicochemical factors of the environment. Thus certain macromolecular anions that do occur in the tissues can inhibit the activity and even the production of hyaluronidase, as well as of other staphylococcal products. Of even greater interest perhaps, is the fact that a strain of *Staphylococcus* that has partly lost the ability to produce hyaluronidase may not recover its full potential ability in this regard until it has multiplied in the proper medium for many generations.

The readiness with which staphylococci exhibit these genotypic and phenotypic changes makes it extremely difficult to define a strain in terms of its pathogenic potentialities. Even the recovery of a culture directly from a lesion may at times yield erroneous or at least incomplete information concerning the characteristics of the staphylococci that were initially responsible for the disease. It seems possible, for example, that the organisms that initiate the disease process may become profoundly altered in some of their characteristics during sequestration in an abscess. In other words, a culture directly recovered from an abscess may have lost temporarily some of the very properties that had first endowed it with invasive power.

Nowhere in this monograph is there any discussion of acquired protective immunity to staphylococcal infection, except for a few limited statements concerning antitoxic immunity. This neglect reflects, of course, the lack of knowledge and of experimental work on the subject. It is probable that studies on acquired immunity would be greatly facilitated if the constituents or products of staphylococci responsible for their ability to establish infection and to cause lethal damage had been properly identified. Until this has been done, attempts at vaccination are likely to remain empirical and probably fruitless, since there is as yet no guide to determine the orientation to be given to the immunization process.

Determinants of Host Susceptibility

Throughout this publication, there is an implicit or explicit recognition by most of the authors that the susceptibility of human beings to staphylococcal infections can be affected profoundly by nonspecific factors. Unfortunately this recognition has not been supplemented by either experimental facts or working hypotheses. I shall take the liberty, therefore, to present in a very abbreviated and dogmatic manner some conclusions pertaining to this problem that have been reached as a result of work done in my laboratory in collaboration with R. W. Schaedler and J. Maclean Smith. Our approach to the problem of susceptibility to infection has been to study the effect of a number of nonspecific factors both on the life expectancy of mice infected by the intra-

venous route with staphylococci and on the survival and multiplication of the cocci in their organs. In brief we have found that the susceptibility of mice to a standard infection can be increased or decreased at will, and that the changes in susceptibility can occur very rapidly, often in a matter of hours. Moreover, these changes are often completely reversible within short periods of time. Only a few of the nonspecific factors that can bring about these changes in susceptibility need be mentioned here. They include nutritional and other metabolic disturbances (for example, those associated with the acute phase of starvation, uncontrolled diabetes, and von Gierke's disease), a variety of toxemias (as caused, for example, by the injection of bacterial endotoxins), subclinical allergic reactions, and the like. Changes in the vascular bed, in the inflammatory response, and in the biochemical environment prevailing at the site of infection, probably play a role as important as that of the specific immunological state in determining the outcome of the infectious process.

The reversibility of the changes in susceptibility and the multiplicity of the nonspecific factors that can bring them about point to the highly dynamic character of staphylococcal infections. The tissue environment determines the outcome of infection, not only through metabolic reactions that affect quantitatively the survival and multiplication of the staphylococci, but also, as we have seen, by changing qualitatively the very characteristics of the microorganisms that are of importance in pathogenesis.

The analysis of these complex dynamic relationships will require new concepts and new techniques to supplement those of classical immunology. In addition to their practical importance, staphylococcal infections provide excellent material for the investigation of these problems. Because they illustrate in such an obvious manner that the physiological state of the host is as important a determinant of disease as is the virulence of the microorganism, the study of staphylococcal infections will very likely throw light on the mechanisms that control the dynamic and ever-changing interrelationships between host and parasite.

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